Table of Contents

Experimental Procedures

Materials and Methods

All commercially available solvents and reagents were used without purification. PNIPAM dendrimer (Mn5500) was purchased from Sigma Aldrich Inc (USA). Thiodiglycolic anhydride was purchased from Fisher. N-Acetylneuraminic acid (Neu5Ac) and cytidine 5'-triphosphate (CTP) were purchased from Carbosynth Limited. Sugar nucleotides uridine 5'-diphospho-galactose (UDP-Gal),[1] UDP-N-acetylglucosamine (UDP-GlcNAc),[2] UDP-N-acetylgalactosamine (UDP-GalNAc),[2] and guanosine 5'-diphospho-L-fucose (GDP-Fuc)[2] were prepared as described previously. *Helicobacter pylori* β1,3-*N*-acetylglucosaminyltransferase (HpLgtA),[3] *Neisseria meningitides* β1,4-galactosyltransferase (NmLgtB),[3] *Neisseria meningitidis* CMP-sialic acid synthetase (NmCSS),[4] *Pasteurella multocida* multifunctional α2,3-sialyltransferase 1 M144D mutant (PmST1 M144D),[5] *Campylobacter jejuni* β1,4-*N*-acetyl-galactosaminyltransferase (CgtA),[6] *Campylobacter jejuni* β1,3-galactosyltransferase mutant (CgtB),[7] *Helicobacter mustelae* α1,2 -fucosyltransferase,[8] *Helicobacter mustelae* α1,3-*N*-acetyl-galactosaminyltransferase (BgtA),[9] and human blood group B glycosyltransferase (GTB)[10] were expressed and purified as described previously.

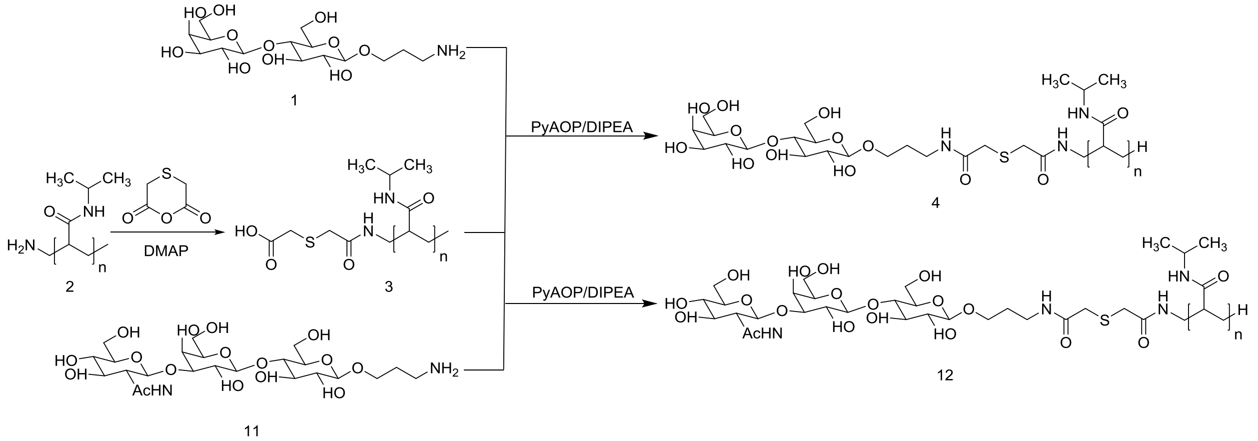
^{1}H-NMR and ^{13}C-NMR spectra were recorded by using a Bruker 400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C) or a Bruker 600 spectrometer (600 MHz for ^{1}H, 150 MHz for ^{13}C). All ^{1}H Chemical shifts (in ppm) were assigned in reference to $CDCl_3$ (δ= 7.24 ppm), MeOD (δ= 4.87 ppm), and D_2O (δ = 4.79 ppm) and all ^{13}C NMR spectra were calibrated with $CDCl_3$ (δ = 77.00 ppm). HPLC was performed with a Shimadzu Prominence UFLC. Column: Waters XBridge BEH amide column, 130 Å, 5 μm, 4.6 mm × 250 mm. A semi-preparative Waters XBridge BEH amide column (130 Å, 5 μm, 10 mm × 250 mm) was used for purification. Solvent A: 100 mM ammonium formate, pH 3.2. Solvent B: Acetonitrile. Semi-preparative Agilent Eclipse XDB-C18, 5 μm, 9.4 × 250 mm. Aeris PEPTIDE 3.6 μm CB-C18, 4.6 mm x 250 mm. Solvent A: 0.1% TFA Water. Solvent B: 0.1% Acetonitrile. High-resolution electrospray ionization mass spectrometry (ESI-HRMS) experiment was performed on an LTQ-Orbitrap Elite mass spectrometer (Thermo Fisher).

Automated synthesis was performed on a Liberty Blue peptide synthesizer (Figure 2, CEM) according to the basic manufacture's manual and software for controlling operation with a designated program (Figure 4A) and sequence for the synthesis of the present target compounds.

Supplementary Text

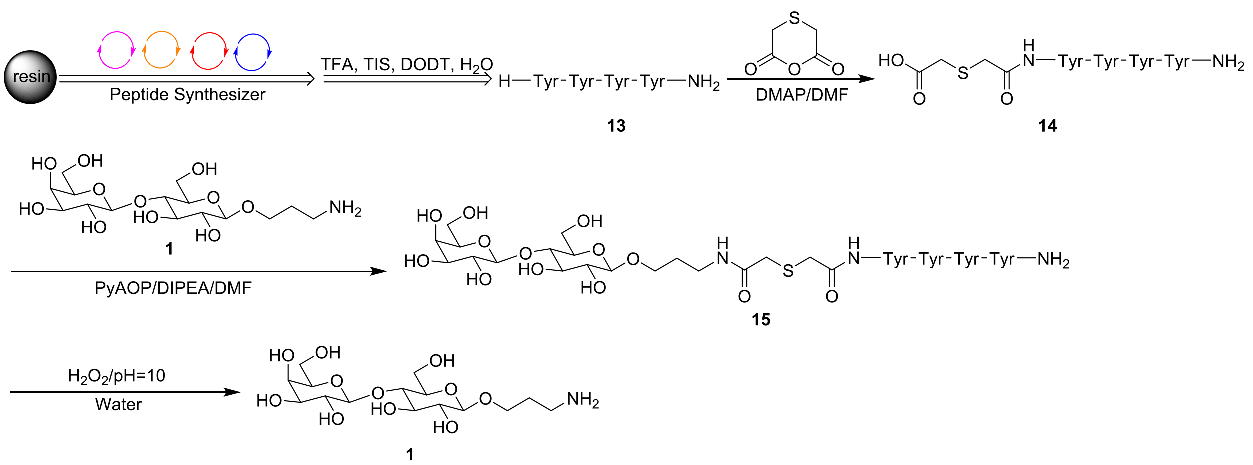
Synthesis.

Scheme S1



Compound **4** was prepared from PNIPAM. In detail, PNIPAM (550 mg, 0.1 mmol) was dissolved in DMF (10 ml). Thiodiglycolic anhydride (132 mg, 1 mmol) and DMAP (122 mg, 1 mmol) were added to the solution, and the reaction mixture was stirred at room temperature overnight. After the reaction completed the solvent was removed by high *vacuo* and the product purified by dialysis (MWCO 1000) to obtain compound **3**. Compound **3** and compound **1** (80 mg, 0.2 mmol) were dissolved in DMF (10 ml). PyAOP (522 mg, 1 mmol) and DIPEA (129 mg, 1 mmol) were added to the solution, and the reaction mixture was stirred at room temperature overnight. The solvent was removed by high *vacuo* and the product purified by dialysis (MWCO 1000) to obtain compound **4**. Compound **12** was prepared from PNIPAM and compound **11** using the protocol as described for the synthesis of compound **4.** The sugar loading efficiency is in the range of 72% (Figure S2).

Scheme S2



Compound **13** was prepared with the peptide synthesizer in 4 cycles. In detail, resin (333 mg, 0.25 mmol) was added to the reaction vessel and all the required reagents were loaded into the tubes. The following procedures were performed by the program on the automated synthesizer. The resin was swelled for 300 s with DMF (10 ml). For the first coupling cycle, 5 ml piperidine in the DMF (v/v=20%) was added to the reaction vessel and the mixture was keep at 50 ℃ for 2 min to deprotect the Fmoc group. The operation was repeated to ensure complete deprotection. Then the resin was washed with DMF (3×5 ml). Tyr solution (5 ml), DIPEA (2 ml), HBTU (2 ml) were added to the reaction vessel to form a solution

(9 ml) as below: (0.25 mmol resin, 1 mmol Tyr, 1 mmol DIPEA and 1 mmol HBTU). The mixture was kept at 50 ℃ for 10 min. This operation was repeated. After 4 cycles, the peptide bound resin was transferred to a tube and 4 ml cleavage cocktail (TFA:H_2O:TIS:DODT=92.5:2.5:2.5:2.5) was added to the tube to give a crude product. The final product was purified by HPLC (acetonitrile, 15%-70%, 40min). ^{1}H-NMR (400 MHz, MeOD), δ 7.051-6.648 (m, 16H), 4.548-4.442 (m, 3H), 3.926 (dd, J_1 = 4.8 Hz, J_2 = 8.4 Hz 1H), 3.083-2.700 (m, 8H). ^{13}C NMR (101 MHz, MeOD) δ 174.27, 171.60, 171.45, 168.30, 156.86, 155.96, 155.90, 130.24, 130.06, 130.03, 129.89, 127.52, 127.38, 127.32, 124.54, 115.49, 114.90, 114.86, 55.09, 54.56, 54.26, 36.74, 36.65, 36.43. HRMS (ESI): m/z $[M +H]^+$calcd for $C_{36}H_{39}N_5O_8$ 670.2872; found: 670.2829.

Compound **14** was prepared from compound **13**. In detail, compound **13** (67 mg, 0.1 mmol) was dissolved in DMF (2 ml). Thiodiglycolic anhydride (66 mg, 0.5 mmol) and DMAP (61 mg, 0.5 mmol) were added to the solution and the reaction mixture was stirred at room temperature overnight. The solvent was removed by high *vacuo*, and the product was purified by HPLC (acetonitrile, 15%-70%, 30min) to get compound **14** (43 mg) in 53.6% yield regarding compound **13**. ^{1}H-NMR (400 MHz, MeOD), 7.04-6.64 (m, 16H), 4.46-4.40 (m, 4H),3.32-2.68 (m, 12H). ^{13}C NMR (101 MHz, MeOD) δ 173.07, 171.11, 170.78, 170.70, 170.26, 169.04, 154.35, 154.27, 128.48, 128.46, 128.40, 126.20, 125.88, 125.87, 125.76, 113.41, 113.36, 54.04, 53.90, 53.68, 53.22, 34.96, 34.86, 34.76, 34.71, 33.43, 32.03. HRMS (ESI): m/z $[M +H]^+$calcd for $C_{40}H_{43}N_5O_{11}S$ 802.2753; found: 802.2744.

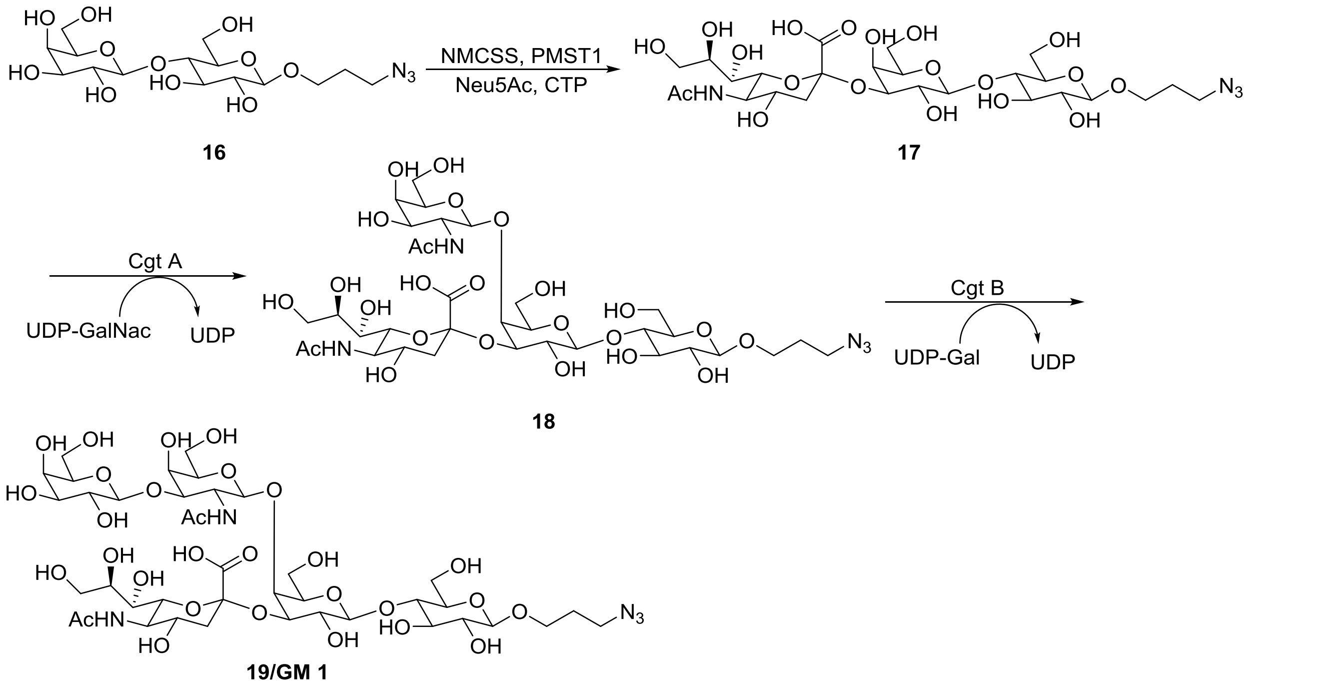
Compound **15** was prepared from compound **14**. In detail, compound **14** (40 mg, 0.05 mmol) and compound **1** (60 mg, 0.15 mmol) were dissolved in DMF (10 ml). PyAOP (261 mg, 0.5 mmol) and DIPEA (65 mg, 0.5 mmol) were added to the solution, and the reaction mixture was stirred at room temperature overnight. The solvent was removed by high *vacuo*, and the product was purified by HPLC (acetonitrile, 3%-40%, 30min) to get compound **15** (21 mg) in 35.5% yield regarding compound **14**. ^{1}H-NMR (400 MHz, MeOD), δ 7.964-6.835 (m, 8H), 6.610-6.562 (m, 8H), 4.380-4.326 (m, 4H), 4.265-4.246 (d, *J* = 7.6 Hz, 1H), 4.204-4.185 (d, *J* = 7.6 Hz, 1H), 3.823-3.317 (m, 12H), 3.208-3.125 (m, 6H), 2.970-2.591 (m, 10H), 1.695-1.665 (m, 2H). HRMS (ESI): m/z $[M +H]^+$ calcd for $C_{55}H_{70}N_6O_{21}S$ 1183.4388; found: 1183.4331.

Sugar release efficiency was determined using compound **15**. In detail, 1 mg of compound **15** was dissolved in 1 M H_2O_2 solution at pH=10. The release efficiency was monitored by HPLC (acetonitrile, 3%-90%, 40min) at time point intervals of 1h, 2h, 4h, and 12h. More than 80% sugar was released from compound **15** (Figure S1). All reactions were run in triplicate.

Enzyme activity test. The enzyme activity for the sugar bound PNIPAM was tested with PmST1. In detail, a reaction mixture with a final volume of 100 μl containing 50 mM Tris-HCl, 1mM of Lactose β-pNP, 10 mM of Neu5Ac, 10 mM of CTP, 5 mM of Mg^{2+}, 1×10^{-3} units of NmCSS, 1×10^{-4}, 2×10^{-4}, 3×10^{-4}, 4×10^{-4}, and 5×10^{-4} units of PmST1 was incubated at 25°C for 30 min. The reaction was quenched by adding an equal volume of ethanol and freezing at -80 °C for 30 min. The reaction was monitored by an analytical GL Science Inertsil ODS-4 column (100 Å, 5 µm, 4.6 mm × 250 mm) with UV detector (260 nm). The running solvents were solvent A (H_2O with 0.1% TFA) and solvent B (acetonitrile with 0.1% TFA). The running condition was a gradient elution with solvent B linear increased from 5% to 10% within 25 min, with a total flow rate of 1 mL/min. All reactions were run in triplicate. The reactivity of sugar bound PNIPAM was tested with Lactose-PNIPAM (compound **4**) using the same conditions. The reaction was monitored by Aeris PEPTIDE 3.6 µm CB-C18, 4.6 mm x 250 mm with UV detector (210 nm). The running solvents were solvent A (H_2O with 0.1% TFA) and solvent B (acetonitrile with 0.1% TFA). The running condition was gradient elution with solvent B being held at 20% for 5 min, then increasing linearly to 45% over 2 min, then holding at 45% for 5 mins, then increasing linearly to 60% over 2 min, then holding at 60% for 2 mins, then increasing linearly to 80% over 2 min, then holding at 80% for 13 mins with a total flow rate of 1 mL/min.

Time optimization was performed with the same substrate Lactose-pNP and Lactose-PNIPAM (compound **4**). In detail, a reaction mixture in a final volume of 100 µl containing 50 mM Tris-HCl, 1mM of Lactose-pNP, 10 mM of Neu5Ac, 10 mM of CTP, 5 mM of Mg^{2+}, 1×10^{-3} units NmCSS, and 3×10^{-4} units of PmST1 was incubated at 25°C for 30 min (1 h, 2 h, 3 h and 6 h). The reaction was quenched by adding an equal volume of ethanol and freezing at -80 °C for 30 min. The reaction was monitored by an analytical GL Science Inertsil ODS-4 column (100 Å, 5 µm, 4.6 mm × 250 mm) with UV detector (260 nm). The running solvents were solvent A (H_2O with 0.1% TFA) and solvent B (acetonitrile with 0.1% TFA). The running condition was gradient elution with solvent B linearly increasing from 5% to 10% over 25 mins, with a total flow rate of 1 mL/min. All reactions were run in triplicate. The reactivity of sugar bound PNIPAM was tested by Lactose-PNIPAM (compound **4**) with the same conditions. The reaction was monitored by Aeris PEPTIDE 3.6 µm CB-C18, 4.6 mm x 250 mm with UV detector (210 nm). The running solvents were solvent A (H_2O with 0.1% TFA) and solvent B (acetonitrile with 0.1% TFA). The running condition was gradient elution with solvent B at 20% for 5 min, then increasing linearly to 45% over 2 min, then holding at B 45% for 5 mins, then increasing linearly to 60% over 2 min, then holding at 60% for 2 mins, then increasing linearly to 80% over 2 min, then holding at 80% for 13 mins with a total flow rate of 1 mL/min.

Scheme S3

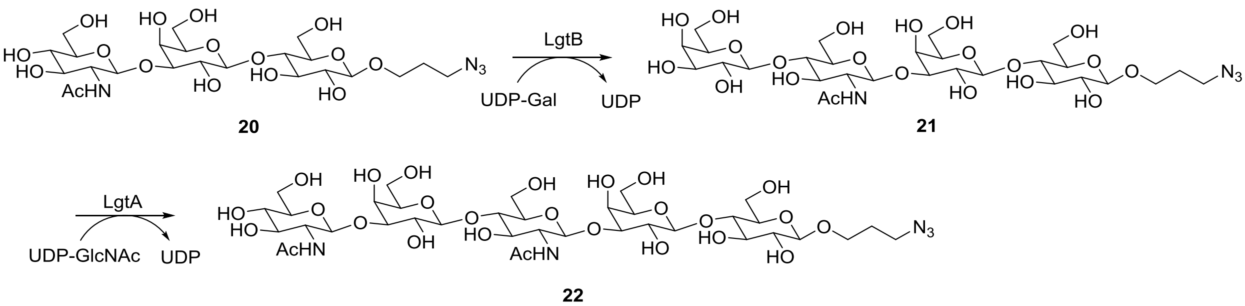


Compound **17** was prepared from compound **16** by using NmCSS and PmST1. In detail, a reaction mixture with a final volume of 10 ml containing 50 mM Tris-HCl, 42.5 mg of 10 (0.1 mmol), 15 mM of Neu5Ac, 15 mM of CTP, 5 mM of Mg^{2+}, 7.4 mg of NmCSS, and 1.6 mg of PmST1 was incubated at 25°C to allow the formation of compound **17.** After 3 hours, the reaction was monitored by TLC ($EtOAc/MeOH/H_2O/HOAc$=5:2:1.4:0.4). An equal volume of ethanol was added to remove proteins and the solution was concentrated *in vacuo*. After purification by Bio-Gel P-2 column 60 mg of compound **17** was obtained in 83.8% yield regarding compound **16**. ^{1}H-NMR (400 MHz, D_2O), δ 4.44 (d, J = 8.0 Hz, 1H), 4.39 (d, J = 8 Hz, 1H), 4.03 (dd, J_1 = 2.8 Hz, J_2 = 6 Hz 1H), 3.92-3.45 (m, 19H), 3.37 (t, J = 6.6 Hz, 2H), 3.23 (t, J = 4.4 Hz 1H), 2.68 (dd, J_1 = 4.4 Hz, J_2 = 12.4 Hz, 1H), 1.93 (s, 3H), 1.84 (m, 2H), 1.73 (t, J = 12.4, 1H). ^{13}C NMR (100 MHz, D_2O), δ 174.97, 173.86, 102.60, 102.10, 99.75, 78.18, 75.43, 75.13, 74.73, 74.30, 72.83, 72.76, 71.73, 69.32, 68.32, 68.04, 67.41, 67.32, 62.52, 60.98, 59.99, 51.63, 47.82, 39.58, 28.18, 21.99. HRMS (ESI): m/z $[M+Na]^+$ calcd for $C_{26}H_{44}N_4O_{19}$ 739.2492; found: 739.2434.

Compound **18** was prepared from compound **17** by using CgtA. In detail, a reaction mixture with a final volume of 8.4 ml containing 50 mM Tris-HCl, 10 mM trisaccharide (60mg), 15 mM of UDP-GalNAc, 5 mM of Mg^{2+}, and 2.1 mg of CgtA was incubated at 25°C to allow the formation of tetrasaccharide. After 6 hours, the reaction was monitored by TLC ($EtOAc/MeOH/H_2O/HOAc$=5:2:1.4:0.4). An equal volume of ethanol was added to remove proteins and the solution was concentrated *in vacuo*. After purification by using Bio-Gel P-2 column. 52 mg of compound **18** was obtained in 67.5% yield regarding compound **17**. ^{1}H-NMR (400 MHz, D_2O), δ 4.74 (d, J = 8.8 Hz, 1H), 4.53 (d, J = 8.0 Hz, 1H), 4.49 (d, J = 8 Hz, 1H), 4.16 (m, 2H), 4.02-3.57 (m, 22H), 3.48-3.27 (m, 5H), 2.68 (dd, J_1 = 4.4 Hz, J_2 = 12.4 Hz, 1H), 2.02 (m, 6H), 1.95 (m, 3H). ^{13}C NMR (100 MHz, D_2O), δ 174.97, 174.79, 174.05, 102.71, 102.54, 102.06, 101.58, 78.53, 77.11, 74.69, 74.32, 74.26, 73.96, 73.01, 72.68, 72.23, 71.21, 69.96, 68.66, 67.95, 67.72, 67.31, 62.78, 61.11, 60.49, 60.04, 52.28, 51.53, 48.06, 47.82, 36.87, 28.18, 22.55, 21.99. HRMS (ESI): m/z [M +Na]$^+$calcd for $C_{34}H_{57}N_5O_{24}$ 942.3285; found: 942.3248.

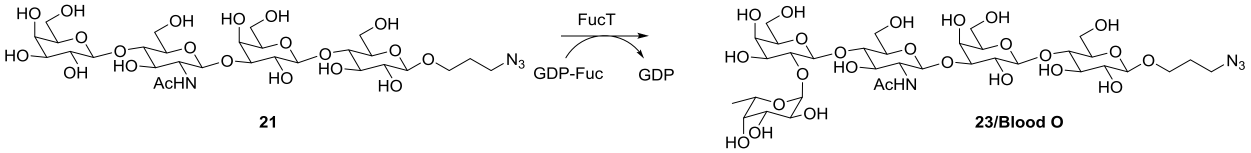
Compound **19** was prepared from compound **18** by using CgtB. In detail, a reaction mixture with a final volume of 5.6 ml containing 50 mM Tris-HCl, 10 mM tetrasaccharide (52mg), 15 mM of UDP-Gal, 5 mM of Mg^{2+}, and 1.8 mg of CgtB was incubated at 25°C to allow the formation of compound **19**. After 6 hours, the reaction was monitored by TLC (EtOAc/MeOH/H_2O/HOAc=5:2:1.4:0.4). An equal volume of ethanol was added to remove proteins and the solution was concentrated *in vacuo*. 48 mg of compound **19** was obtained in 79% yield regarding compound **18**. ^{1}H-NMR (400 MHz, D_2O), δ 4.67 (d, *J* = 8.8 Hz, 1H), 4.43 (m, 3H), 4.04 (m, 3H), 3.94 (m, 3H), 3.79-3.32 (m, 27H), 3.26-3.16 (m, 2H), 2.56 (m, 1H), 1.91-1.77 (m, 9H). ^{13}C NMR (151 MHz, D_2O) δ 175.02, 174.76, 174.11, 104.73, 102.58, 102.49, 102.10, 101.63, 80.31, 78.60, 77.13, 74.87, 74.73, 74.37, 74.34, 74.32, 74.05, 73.07, 72.72, 72.48, 72.25, 70.67, 70.00, 68.70, 68.57, 68.00, 67.89, 67.35, 62.81, 61.08, 60.92, 60.59, 60.09, 51.58, 51.17, 48.11, 47.87, 36.92, 28.22, 22.57, 22.03. HRMS (ESI): m/z [M +Na]$^+$ calcd for $C_{40}H_{67}N_5O_{29}$ 1104.3814; found: 1104.3766.

Scheme S4



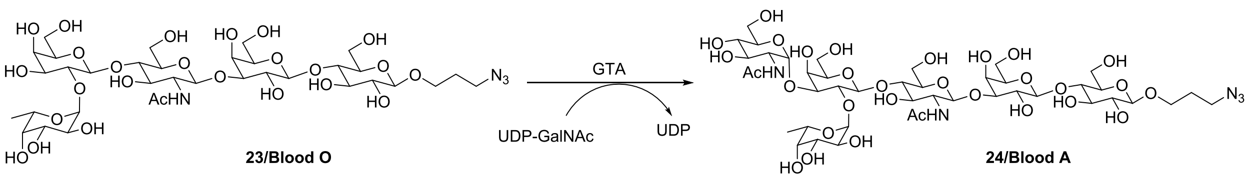
Compound **22** was prepared from compound **21**[11] using LgtA. In detail, a reaction mixture in a final volume of 2 ml mixture containing 50 mM Tris-HCl (pH 8.0), 15.8 mg of compound **21** (10 mM), 5 mM of Mg^{2+}, 15 mM of UDP-GlcNAc, and 0.6 mg of LgtA was incubated at 25°C to allow the formation of compound **22**. After 12 hours, the reaction was monitored by TLC (EtOAc/MeOH/H_2O/HOAc=5:2:1.4:0.4). An equal volume of ethanol was added to remove proteins and the solution was concentrated *in vacuo*. The product was purified by using Bio-Gel P-2 column. 13 mg of compound **22** was obtained in 65.5 % isolated yield regarding **21**. ^{1}H-NMR (400 MHz, D_2O), δ 4.70 (m, 2H), 4.49 (m, 3H), 4.15 (d, *J* = 2.8 Hz, 1H), 3.99-3.44 (m, 31H), 3.32 (m, 1H), 2.03 (s, 6H), 1.94 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 174.90, 102.82, 102.71, 102.05, 81.94, 78.23, 78.05, 75.58, 74.81, 74.71, 74.48, 74.28, 73.48, 72.71, 72.10, 69.91, 69.59, 68.26, 67.30, 60.89, 60.38, 59.96, 59.76, 55.58, 55.07, 47.78, 28.16, 22.08. HRMS (ESI): m/z [M +H]$^+$ calcd for $C_{37}H_{63}N_5O_{26}$ 994.3834; found: 994.3757.

Scheme S5



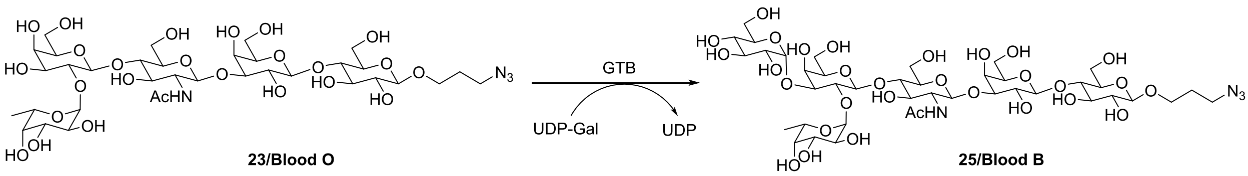
Compound **23** was prepared from compound **21** using FucT from *Helicobacter mustelae*. In detail, a reaction mixture in a final volume of 4 ml mixture containing 50 mM Tris-HCl (pH 8.0), 31.6 mg of compound **21** (10 mM), 5 mM of Mg^{2+}, 15 mM of GDP-Fucose, and 2.5 mg of 1,2-FucT was incubated at 25°C to allow the formation of compound **23**. After 6 hours, the reaction was monitored by TLC (EtOAc/MeOH/H_2O/HOAc=5:2:1.4:0.4). An equal volume of ethanol was added to remove proteins and the solution was concentrated *in vacuo*. The product was purified by using Bio-Gel P-2 column. 32 mg of compound **23** was obtained in 85.5 % isolated yield regarding compound **21**. ^{1}H-NMR (400 MHz, D_2O), δ 5.29 (d, J = 2.4 Hz, 1H), 4.69 (d, J = 8.4 Hz, 1H), 4.54 (d, J = 7.6 Hz, 1H), 4.48 (d, J = 8 Hz, 1H), 4.43 (d, J = 8 Hz, 1H), 4.22 (dd, J_1 = 6.4 Hz, J_2 = 13.2 Hz, 1H), 4.13 (d, J = 3.2 Hz, 1H), 4.01-3.54 (m, 26H), 3.46 (t, J = 6.4 Hz, 3H), 3.31 (m, 1H), 2.02 (s, 3H), 1.92 (m, 2H), 1.21 (d, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ 174.88, 102.86, 102.73, 102.05, 100.17, 99.35, 81.92, 78.24, 76.35, 75.75, 75.19, 75.03, 74.79, 74.71, 74.28, 73.46, 72.73, 72.00, 71.60, 69.92, 69.54, 69.06, 68.26, 68.12, 67.30, 66.88, 61.07, 60.86, 59.97, 59.92, 55.31, 47.79, 28.17, 22.14, 15.26. HRMS (ESI): m/z $[M+Na]^+$ calcd for $C_{35}H_{60}N_4O_{25}$ 959.3439; found: 959.3450.

Scheme S6



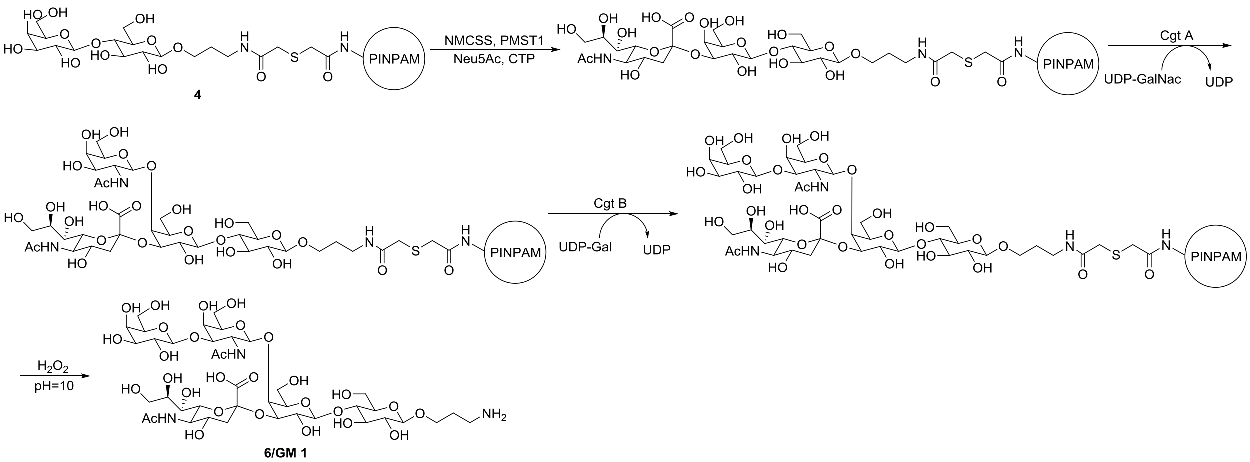
Compound **24** was prepared from compound **23** using BgtA. In detail, a reaction mixture in a final volume of 1.5 ml mixture containing 50 mM Tris-HCl (pH 8.0), 14 mg of compound **23** (10 mM), 5 mM of Mg^{2+}, 15 mM of UDP-GalNAc, and 0.6 mg of BgtA was incubated at 25°C to allow the formation of compound **24**. After 6 hours, the reaction was monitored by TLC (EtOAc/MeOH/H_2O/HOAc=5:2:1.4:0.4). An equal volume of ethanol was added to remove proteins and the solution was concentrated *in vacuo*. The product was purified by using HPLC (water/acetonitrile,75-35, 16min/35min). 10.2 mg of compound **24** was obtained in 59.9 % isolated yield regarding compound **23**. ^{1}H-NMR (600 MHz, D_2O), δ 5.23 (d, J = 2.4 Hz, 1H), 5.06 (d, J = 2.8 Hz, 1H), 4.58 (d, J = 5.6 Hz, 1H), 4.49 (d, J = 5.2 Hz, 1H), 4.37 (d, J = 5.2 Hz, 1H), 4.33 (d, J = 5.6 Hz, 1H), 4.20 (dd, J_1 = 4 Hz, J_2 = 8.8 Hz, 1H), 4.12 (m, 3H), 4.02 (d, J = 2.4 Hz, 1H), 3.87-3.51 (m, 29H), 3.35(m, 3H), 3.20 (m, 1H), 1.92 (s, 3H), 1.91 (s, 3H), 1.81 (m, 2H), 1.13 (d, J = 4.4 Hz, 3H), ^{13}C NMR (151 MHz, D_2O) δ 174.86, 174.73, 102.86, 102.75, 102.06, 99.99, 98.57, 91.25, 81.94, 78.23, 75.86, 75.62, 75.12, 75.08, 74.80, 74.72, 74.29, 72.76, 72.32, 72.08, 71.63, 71.03, 69.95, 69.89, 68.43, 68.26, 67.69, 67.60, 67.30, 66.83, 62.99, 61.26, 61.15, 60.87, 59.97, 59.88, 55.40, 49.45, 47.81, 28.18, 22.19, 21.92, 15.13. HRMS (ESI): m/z [M +Na]$^+$ calcd for $C_{43}H_{73}N_5O_{30}$ 1162.4232; found: 1162.4176.

Scheme S7



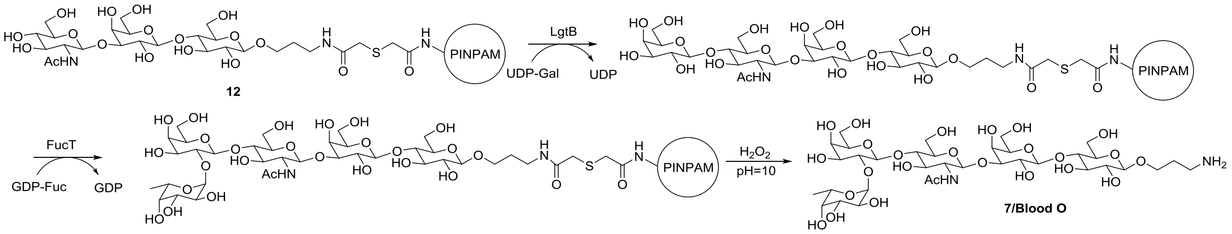
Compound **25** was prepared from compound **23** using GTB from humans. In detail, a reaction mixture in a final volume of 1.5 ml mixture containing 50 mM Tris-HCl (pH 8.0), 14 mg of compound **23** (10 mM), 5 mM of Mg^{2+}, 15 mM of UDP-Gal, and 0.6 mg of GTB was incubated at 25°C to allow the formation of compound **25**. After 6 hours, the reaction was monitored by TLC (EtOAc/MeOH/H_2O/HOAc=5:2:1.4:0.4). An equal volume of ethanol was added to remove proteins and the solution was concentrated *in vacuo*. The product was purified by using HPLC (water/acetonitrile,75-35, 18min/35min). 9.6 mg of compound **25** was obtained in 58.4 % isolated yield regarding compound **23**. ^{1}H-NMR (600 MHz, D_2O), δ 5.29 (d, *J* = 2.8 Hz, 1H), 5.21 (d, *J* = 1.6 Hz, 1H), 4.67 (d, *J* = 5.6 Hz, 1H), 4.59 (d, *J* = 5.2 Hz, 1H), 4.45 (d, *J* = 5.2 Hz, 1H), 4.41 (d, *J* = 5.2 Hz, 1H), 4.27 (m, 2H), 4.17 (t, *J* = 4 Hz, 1H), 4.11 (d, *J* = 2.4 Hz, 1H), 3.98-3.53 (m, 31H), 3.43 (m, 3H), 3.29 (m, 1H), 2.00 (s, 3H), 1.88 (m, 2H), 1.20 (d, *J* = 4.4 Hz, 3H), ^{13}C NMR (151 MHz, D_2O) δ 174.88, 102.87, 102.77, 102.07, 100.05, 98.74, 92.96, 81.97, 78.25, 76.11, 75.84, 75.07, 74.91, 74.82, 74.73, 74.31, 72.78, 72.48, 72.10, 71.64, 71.10, 69.97, 69.45, 69.23, 68.27, 68.01, 67.65, 67.32, 66.79, 63.44, 62.44, 61.23, 61.13, 60.89, 59.99, 59.89, 55.39, 47.83, 28.20, 22.21, 15.15. HRMS (ESI): m/z [M +Na]$^+$ calcd for $C_{41}H_{70}N_4O_{30}$ 1121.3967; found: 1121.3912.

Scheme S8



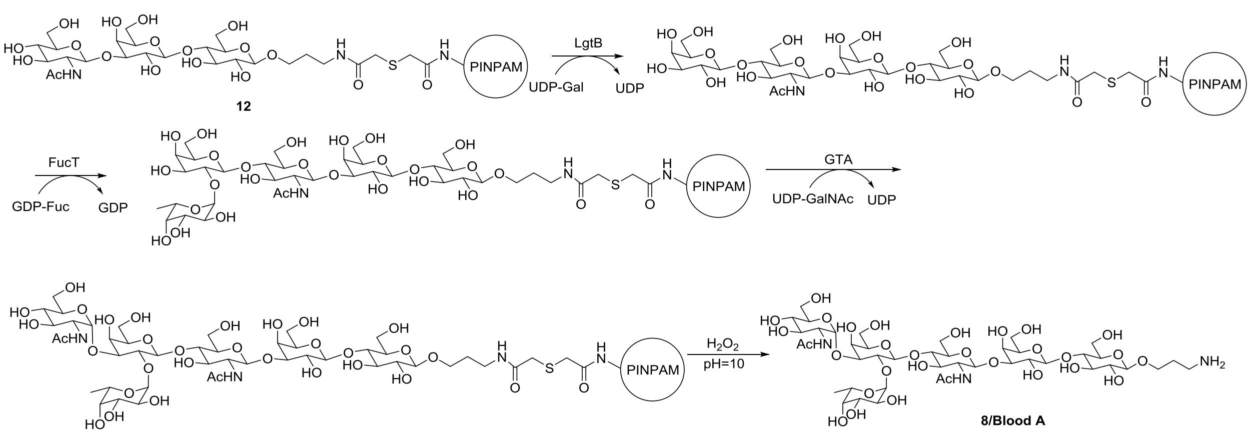
Fully automated enzymatic synthesis of GM 1 on our automated synthesizer. A synthetic program was made for compound **6** that contains three cycles (table 1). Compound **4** was loaded into the reaction vessel and all the enzymes and sugar nucleotide solutions added to the corresponding tubes. The following procedures were performed by the program on the automated synthesizer. For the first glycosylation, steps 1 and 2: Neu5Ac, CTP, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0) and NmCSS, PmST1 (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 15 mM Neu5Ac and CTP, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 7.4 mg of NmCSS, 1.6 mg of PmST1, and 5 mM compound **4**; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 3 h (microwave 10 W, hold 3 h at 25°C, bubble on for 5 s, off for 600 s. This is a special case and all the other glycosyl reactions were kept for 6 h); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). This glycosylation was only done by once and all the others were done by twice to improve the yield. For the second glycosylation, steps 1 and 2: UDP-GalNAc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and CgtA (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-GalNAc, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 2.1 mg of CgtA, and 5 mM resin-bound sugar; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: UDP-GalNAc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and CgtA (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-GalNAc, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 2.1 mg of CgtA, and 5 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). For the third glycosylation, steps 1 and 2: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0) and CgtB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.8 mg of CgtB, and 5 mM resin-bound sugar; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0) and CgtB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.8 mg of CgtB, and 5 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). After three cycles, the resin-bound sugar was transferred to a flask and the sugar released under 1 M H_2O_2 (PH=10). The final compound **6** (20 mg) was purified by HPLC (water/acetonitrile,80-54.2, 35min/45min) in 38% yield from compound **4.** ^{1}H-NMR (400 MHz, D_2O), δ 4.48 (m, 3H), 4.09 (m, 3H), 3.98 (m, 3H), 3.85 (d, *J* = 2 Hz, 1H), 3.81-3.52 (m, 22H), 3.46 (m, 2H), 3.30 (m, 3H), 3.10 (t, *J* = 4.4 Hz, 1H), 2.60 (dd, J_1 = 3.2 Hz, J_2 = 8.8 Hz, 1H), 1.97-1.78 (m, 9H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{40}H_{69}N_3O_{29}$ 1056.4090; found: 1056.4045.

Scheme S9



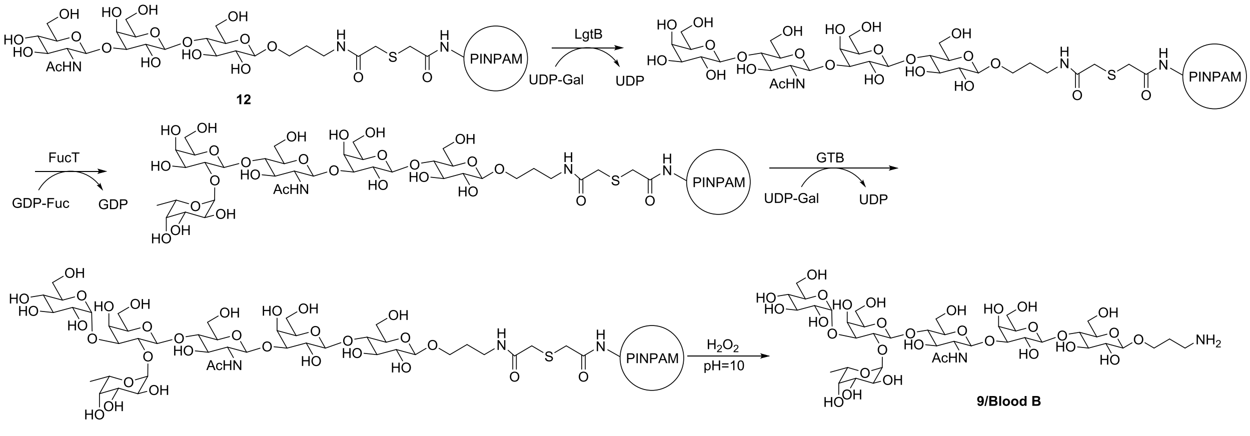
Fully automated enzymatic synthesis of Blood O on our automated synthesizer. A synthetic program was made for synthesizing compound **7** and this program contains two cycles (table 1). Compound **12** was loaded into the reaction vessel and all the enzyme and sugar nucleotide solutions were added to the corresponding tubes. The following procedures were performed by the program on the automated synthesizer. For the first glycosylation, steps 1 and 2: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0) and LgtB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.0 mg of LgtB, and 4 mM compound **12**; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0) and LgtB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.0 mg of LgtB, and 4 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). For the second glycosylation, steps 1 and 2: GDP-Fuc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0) and 1,2-FucT (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM GDP-Fuc, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 2.5 mg of 1,2-FucT, and 4 mM resin-bound sugar; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: GDP-Fuc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0) and 1,2-FucT (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM GDP-Fuc, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 2.5 mg of 1,2-FucT, and 4 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). After two cycles, the resin-bound sugar was transferred to a flask and the sugar released under 1 M H_2O_2 (PH=10). The final compound **7** (13 mg) was purified by HPLC (water/acetonitrile, 80-54.2, 34 min/45 min) in 35% yield from compound **12.** ^{1}H-NMR (600 MHz, D_2O), δ 5.24 (d, *J* = 2 Hz, 1H), 4.64 (d, *J* = 5.6 Hz, 1H), 4.48 (d, *J* = 5.2 Hz, 1H), 4.37 (d, *J* = 5.2 Hz, 1H), 4.15 (m, 1H), 4.07 (m, 1H), 3.98 (m, 2H), 3.82 (m, 2H), 3.74-3.47 (m, 22H), 3.40-3.07 (m, 4H), 1.97 (s, 3H), 1.81 (m, 2H), 1.16 (d, *J* = 4.4 Hz, 3H). HRMS (ESI): m/z $[M+Na]^+$ calcd for $C_{35}H_{62}N_2O_{25}$ 933.3524; found: 933.3592.

Scheme S10



Fully automated enzymatic synthesis of Blood A on our automated synthesizer. A synthetic program was made for compound **8** and this program contains three cycles (table 1). Compound **12** was loaded into the reaction vessel and all the enzyme and sugar nucleotide solutions were added to the corresponding tubes. The following procedures were performed by the program on the automated synthesizer. For the first glycosylation, steps 1 and 2: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and LgtB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.0 mg of LgtB, and 4 mM compound **12**; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and LgtB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.0 mg of LgtB, and 4 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). For second glycosylation, steps 1 and 2: GDP-Fuc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and 1,2-FucT (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM GDP-Fuc, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 2.5 of 1,2-FucT, and 4 mM resin-bound sugar; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: GDP-Fuc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and 1,2-FucT (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM GDP-Fuc, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 2.5 of 1,2-FucT, and 4 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). For the third glycosylation, steps 1 and 2: UDP-GalNAc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and BgtA (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-GalNAc, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.9 mg of GTA, and 4 mM resin-bound sugar; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: UDP-GalNAc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and BgtA (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-GalNAc, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.9 mg of GTA, and 4 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). After three cycles, the resin-bound sugar was transferred to a flask and the sugar released under 1 M H_2O_2 (PH=10). The final compound **8** (14 mg) was purified by HPLC (water/acetonitrile,75-35, 31min/35min) in 29% yield from compound **12.** ^{1}H-NMR (600 MHz, D_2O), δ 5.29 (d, *J* = 2.4 Hz, 1H), 5.11 (d, *J* = 1.6 Hz, 1H), 4.64 (d, *J* = 5.2 Hz, 1H), 4.54 (d, *J* = 5.6 Hz, 1H), 4.45 (d, *J* = 5.6 Hz, 1H), 4.38 (d, *J* = 4.8 Hz, 1H), 4.25 (m, 1H), 4.18 (m, 3H), 4.07 (m, 1H), 3.97-3.83 (m, 9H), 3.76-3.55 (m, 20H), 3.38-3.08 (m, 4H), 1.99 (m, 8H), 1.18 (d, *J* = 4.4 Hz, 3H). HRMS (ESI): m/z $[M +H]^+$ calcd for $C_{43}H_{75}N_3O_{30}$ 1114.4508; found: 1114.4485.

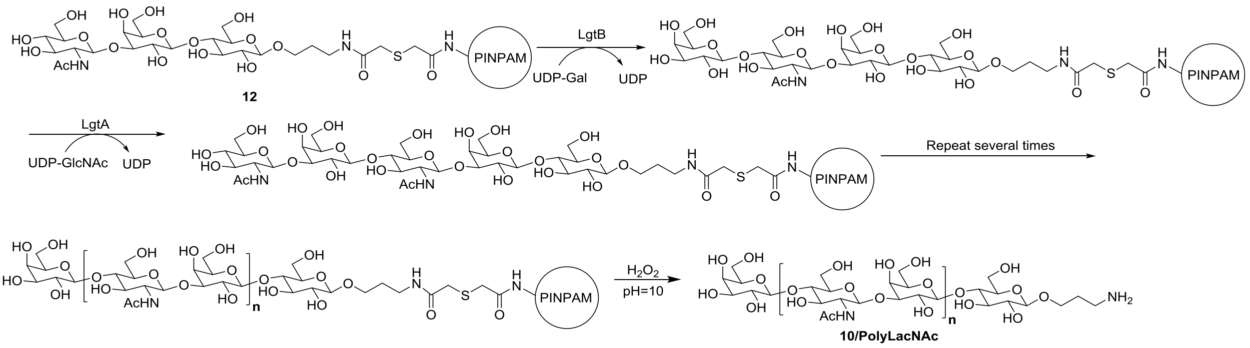
Scheme S11



Fully automated enzymatic synthesis of Blood B on our automated synthesizer. A synthetic program was made for compound **9** and this program contains three cycles (table 1). Compound **12** was loaded into the reaction vessel and all the enzyme and sugar nucleotide solutions were added to the corresponding tubes. The following procedures were performed by the program on the automated synthesizer. For the first glycosylation, steps 1 and 2: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and LgtB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.0 mg of LgtB, and 4 mM compound **12**; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and LgtB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.0 mg of LgtB, and 4 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). For the second glycosylation, steps 1 and 2: GDP-Fuc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and 1,2-FucT (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM GDP-Fuc, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 2.5 mg of 1,2-FucT, and 4 mM resin-bound sugar; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: GDP-Fuc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and 1,2-FucT (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM GDP-Fuc, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 2.5 mg of 1,2-FucT, and 4 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). For the third glycosylation, steps 1 and 2: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and GTB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.8 mg of GTB, and 4 mM resin-bound sugar; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and GTB

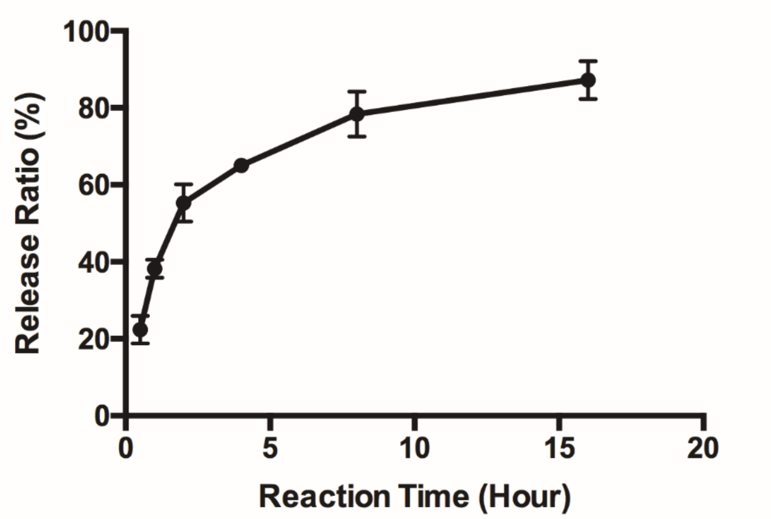
(5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.8 mg of GTB, and 4 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). After three cycles, the resin-bound sugar was transferred to a flask and the sugar released under 1 M H_2O_2 (PH=10). The final compound **9** (13 mg) was purified by HPLC (water/acetonitrile,75-35, 28min/35min) in 27.1% yield from compound **12.** ^{1}H-NMR (600 MHz, D_2O), δ 5.21 (m, 1H), 5.13 (m, 1H), 4.59 (d, J = 5.2 Hz, 1H), 4.52 (d, J = 4.8 Hz, 1H), 4.40 (d, J = 5.2 Hz, 1H), 4.33 (d, J = 5.2 Hz, 1H), 4.19 (m, 2H), 4.08 (m, 1H), 4.03 (m, 1H), 3.86-3.53 (m, 31H), 3.33-3.04 (m, 4H), 2.00-1.90 (m, 5H), 1.20 (m, 3H). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{41}H_{72}N_2O_{30}$ 1073.4243; found: 1073.4229.

Scheme S12



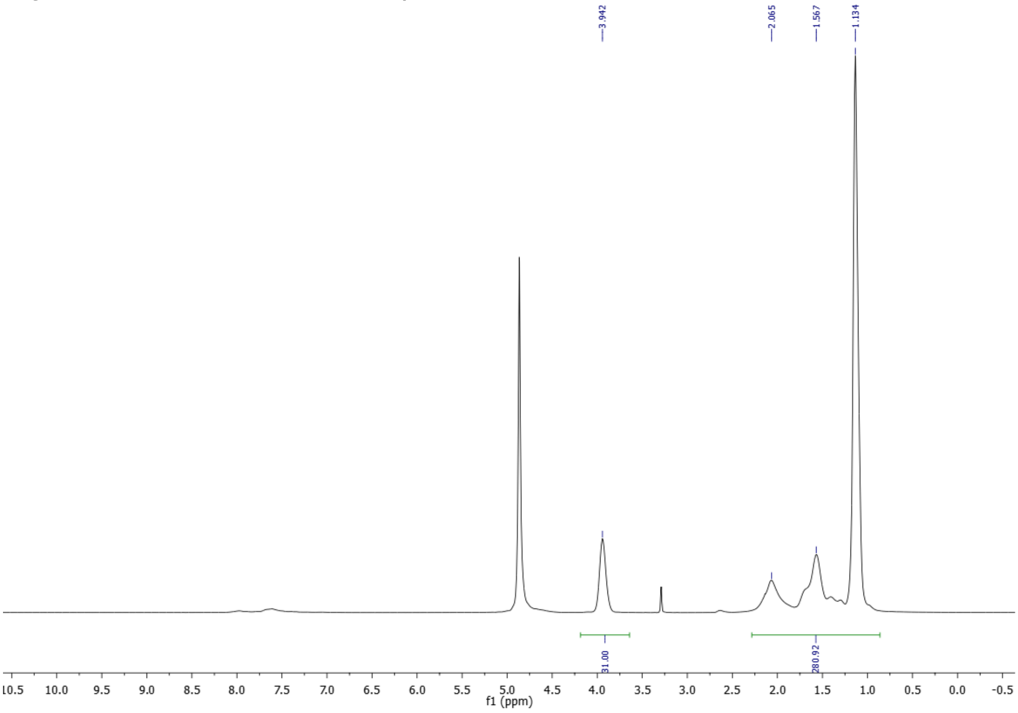
Fully automated enzymatic synthesis of PolyLacNAc on our automated synthesizer. A synthetic program was made for compound **10** and this program contains nine cycles (table 1). Compound **12** was loaded into the reaction vessel and all the enzyme and sugar nucleotide solutions were added to the corresponding tubes. For the first glycosylation, steps 1 and 2: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and LgtB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.0 mg of LgtB, and 4 mM compound **12**; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: UDP-Gal, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and LgtB (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-Gal, 50 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 1.0 mg of LgtB, and 4 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 6 h (microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). For the second glycosylation, steps 1 and 2: UDP-GlcNAc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and LgtA (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-GlcNAc, 50 mM Tris-HCl (pH 8.0), 4 mM Mg^{2+}, 1.7 mg of LgtA, and 4 mM resin-bound sugar; step 3: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 12 h (microwave 10 W, hold 12 h at 25°C, bubble on for 5 s, off for 600 s. This is a special case and all the other glycosyl reactions were kept for 6 h); step 4: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s); steps 5 and 6: UDP-GlcNAc, Mg^{2+} (5 ml) in 100 mM Tris-HCl (pH 8.0), and LgtA (5 ml) were added to the reaction vessel to form a solution (10 ml) as below: 10 mM UDP-GlcNAc, 50 mM Tris-HCl (pH 8.0), 4 mM Mg^{2+}, 1.7 mg of LgtA, and 4 mM resin-bound sugar; step 7: the temperature of the reaction mixture was maintained at 25°C under 10 W of microwave irradiation. Glycosyl reaction was done at 25°C for 12 h (microwave 10 W, hold 12 h at 25°C, bubble on for 5 s, off for 600 s. This is a special one and all the other glycosyl reactions were kept for 6 h); step 8: The temperature of the reaction mixture was quickly brought up to 90°C under 50 W of microwave irradiation. It was kept at 90°C for 10 min and drained to push out all the waste to leave resin-bound sugar only (microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s). To obtain a longer polyLacNAc, these two cycles were repeated. After nine cycles, the resin-bound sugar was transferred to a flask and the sugar released under 1 M H_2O_2 (PH=10). The final compound **10** (8 mg) was purified by HPLC (water/acetonitrile,60-45, 14min/40min) in 9.0% yield from compound **12.** ^{1}H-NMR (600 MHz, D_2O), δ 4.68 (m, 4H), 4.44 (m, 8H), 4.09 (m, 6H), 4.00 (m, 1H), 3.92 (m, 6H), 3.79-3.64 (m, 39H), 3.61-3.46 (m, 14H), 3.28 (m, 1H), 3.10 (m, 3H), 2.01-1.93 (m, 14H). HRMS (ESI): m/z $[M +H+Na]^{2+}$ calcd for $C_{85}H_{144}N_6O_{61}$ 1124.4158; found: 1124.4159.

Figure S1 Lactose release from thioether linker bound peptide

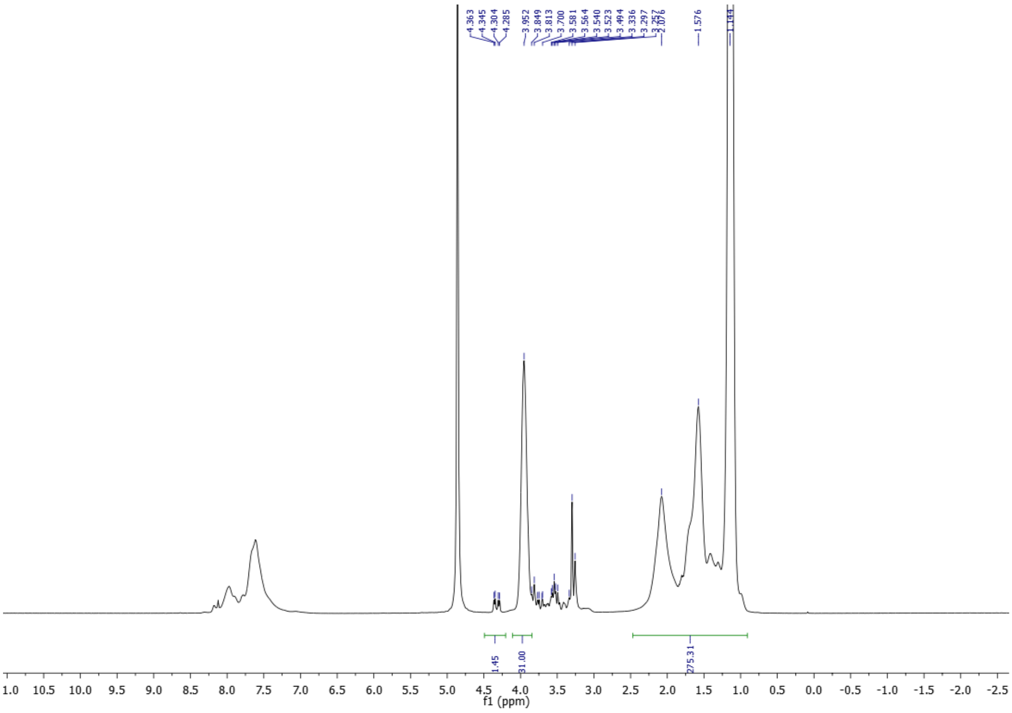


1 mg Lactose bound peptide is treated with 100 mM H_2O_2 at PH=10. After 8 h, about 80% lactose is released from peptide.

Figure S2 400 MHz 1H-NMR spectrum of **PNIPAM** and **Lac-PNIPAM** in MeOD-d4 at 300K



A) proton spectrum of PNIPAM



B) proton spectrum of Lactose linked PNIPAM.

The loading efficiency was calculated by the hydrogen of 1 position on the sugar ring. Comparing to the proton spectrum of PNIPAM, it turns out that the loading efficiency of lactose on the PNIPAM is 72%.

Figure S3 400 MHz NMR spectrum of compound **13** in MeOD-d4 at 300K

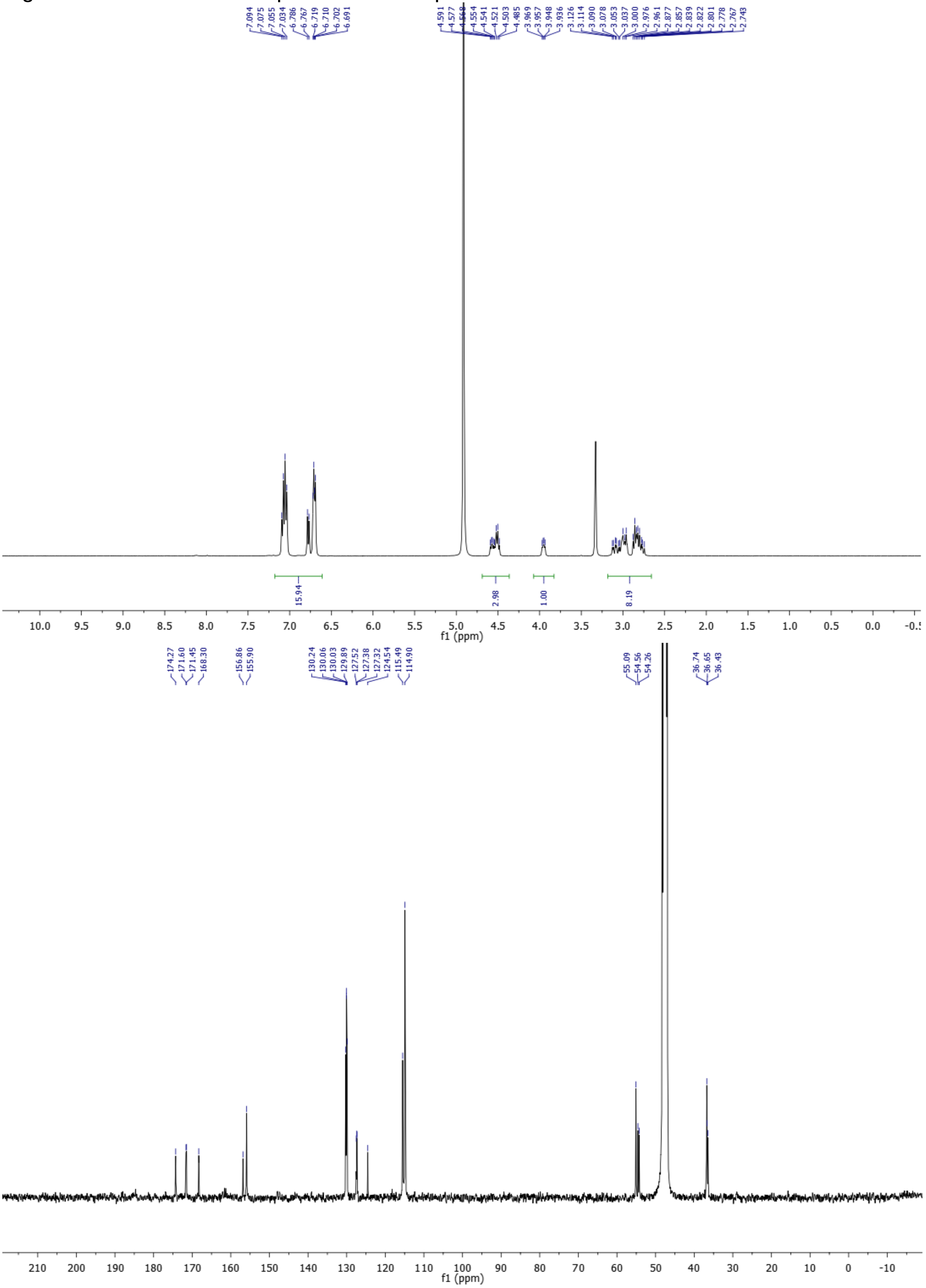


Figure S4 400 MHz NMR spectrum of compound **14** in MeOD-d4 at 300K

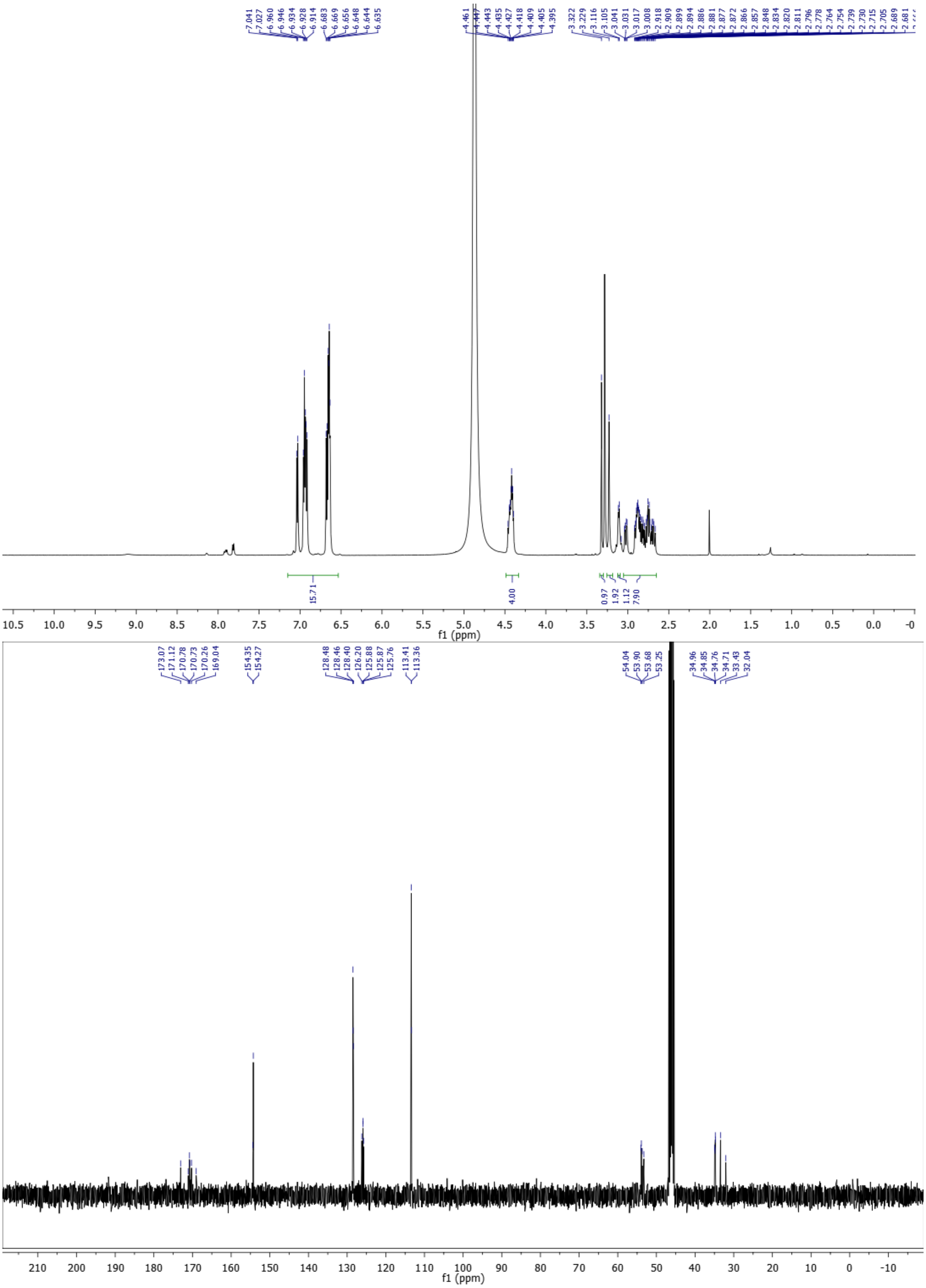


Figure S5 400 MHz NMR spectrum of compound **15** in MeOD-d4 at 300K

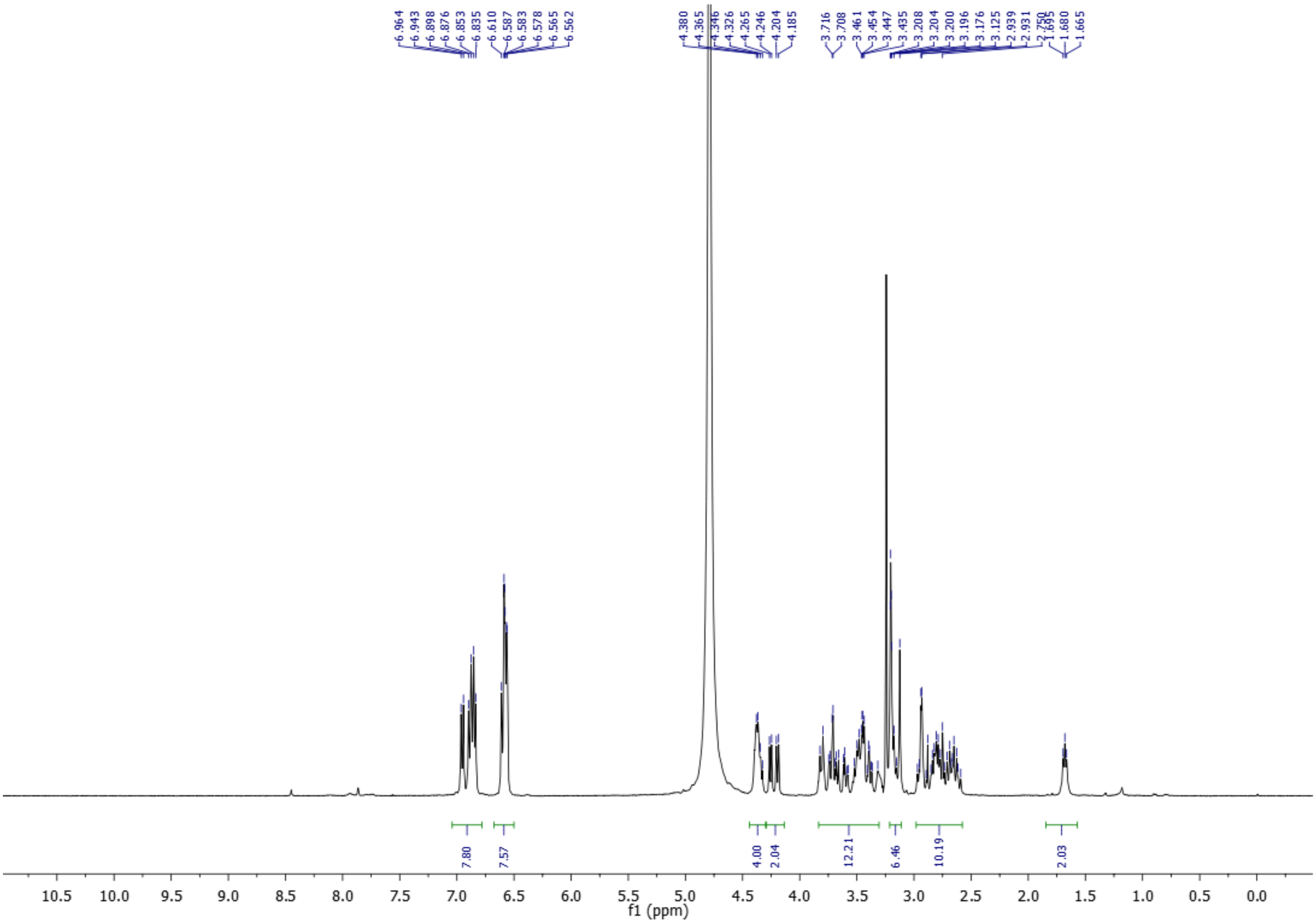
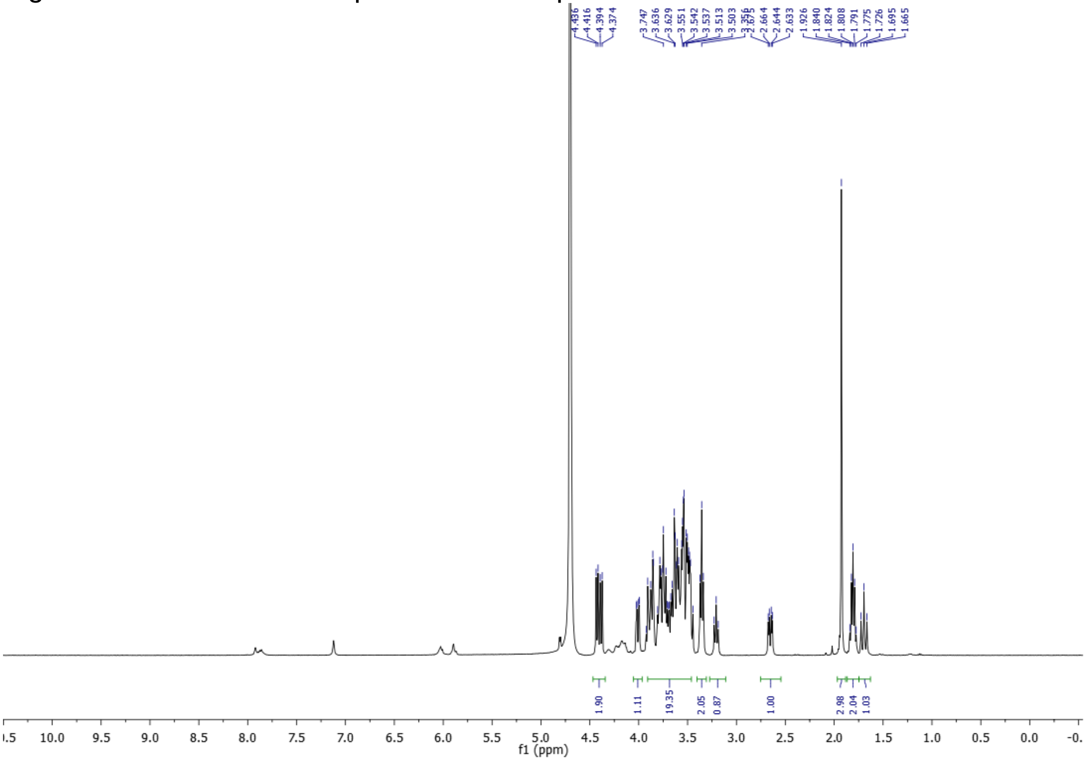
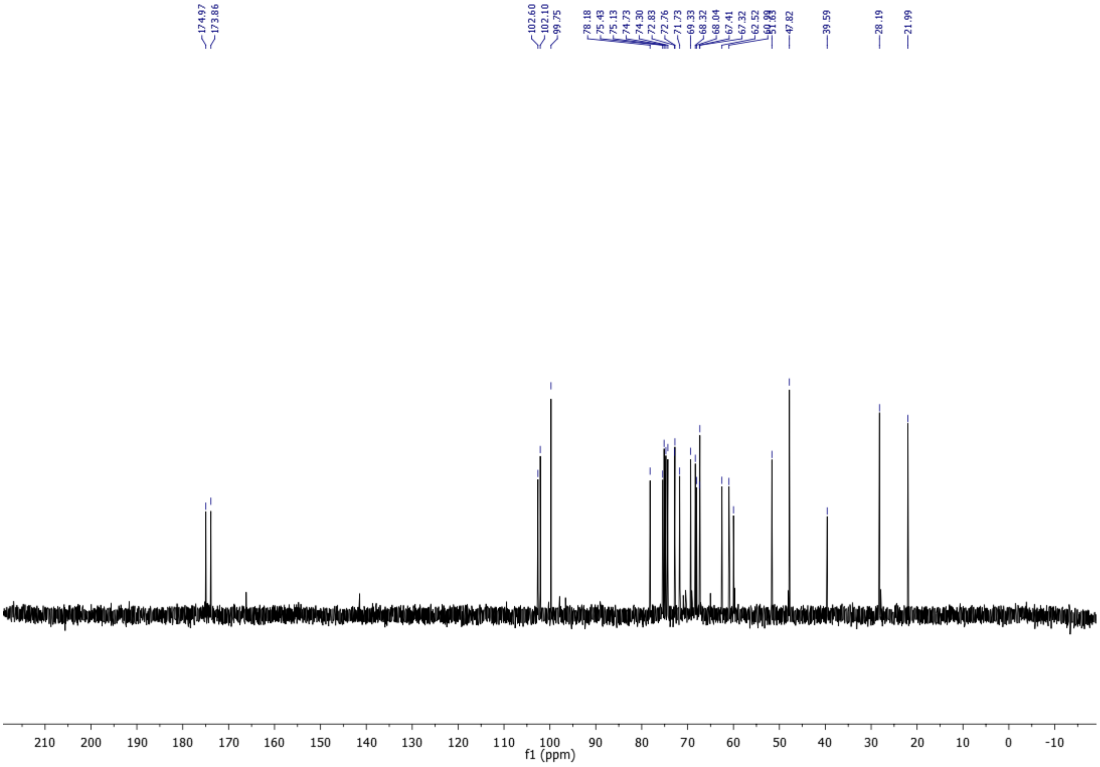


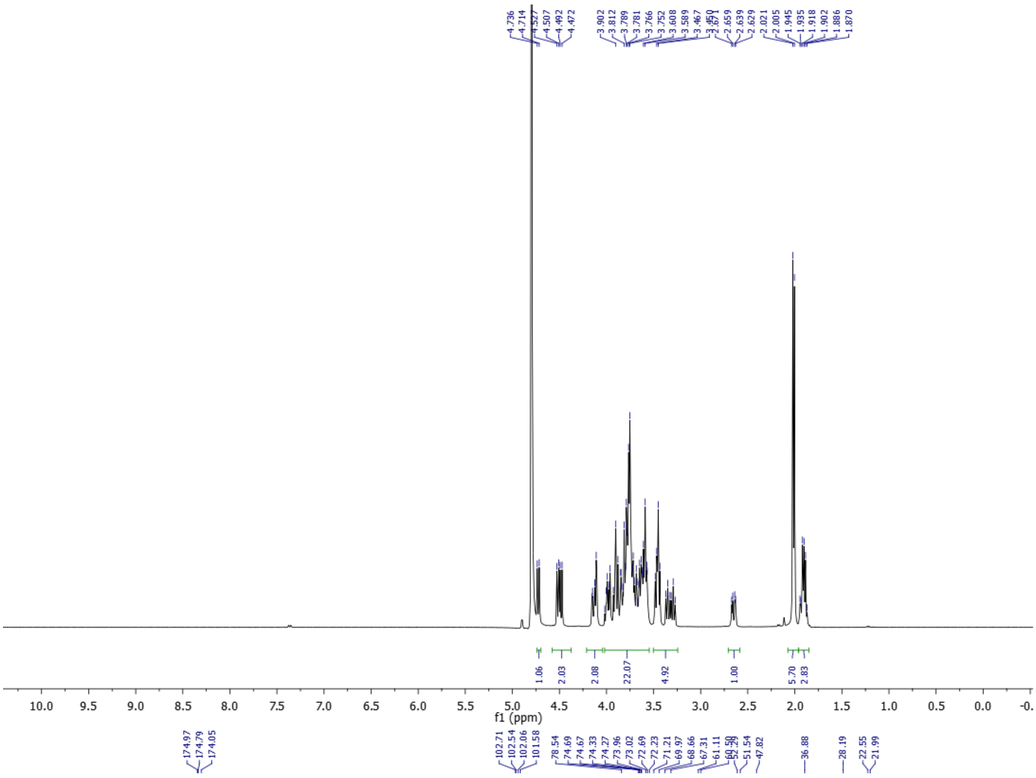
Figure S6 400 MHz NMR spectrum of compound **17** in D_2O at 300K





SUPPORTING INFORMATION

Figure S7 400 MHz NMR spectrum of compound **18** in D_2O at 300K



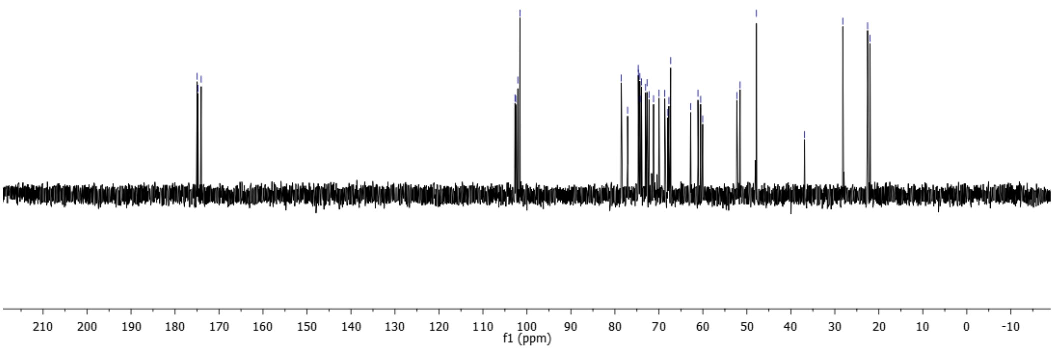
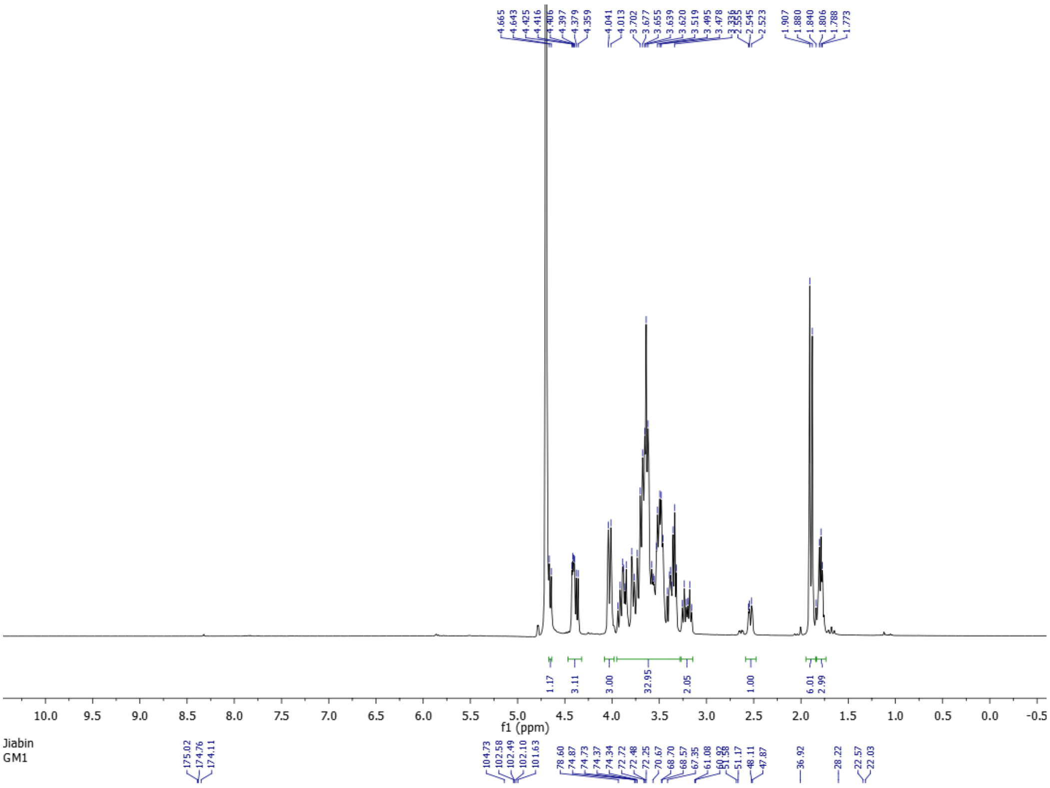


Figure S8 400 MHz NMR spectrum of compound **19** in D_2O at 300K



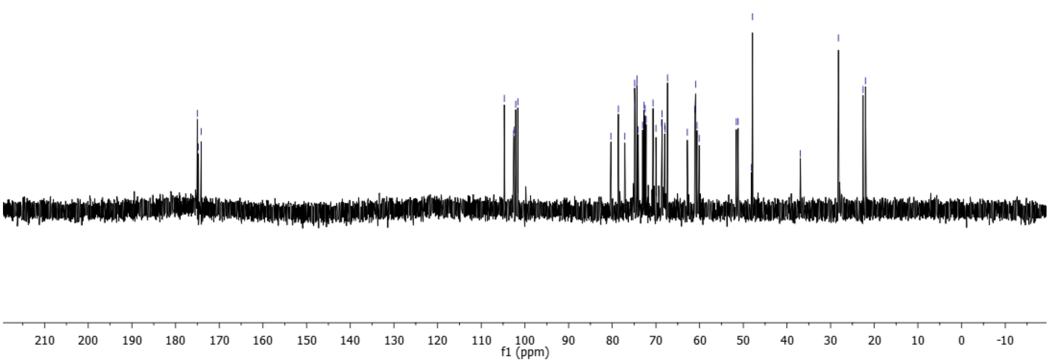
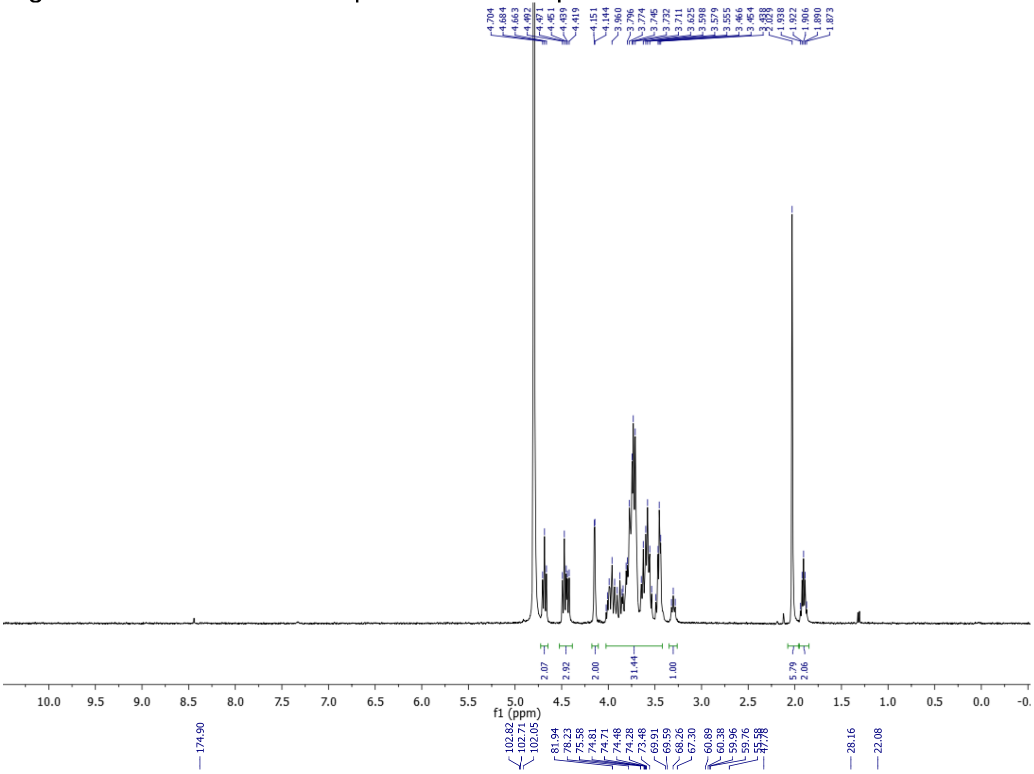


Figure S9 400 MHz NMR spectrum of compound **22** in D_2O at 300K



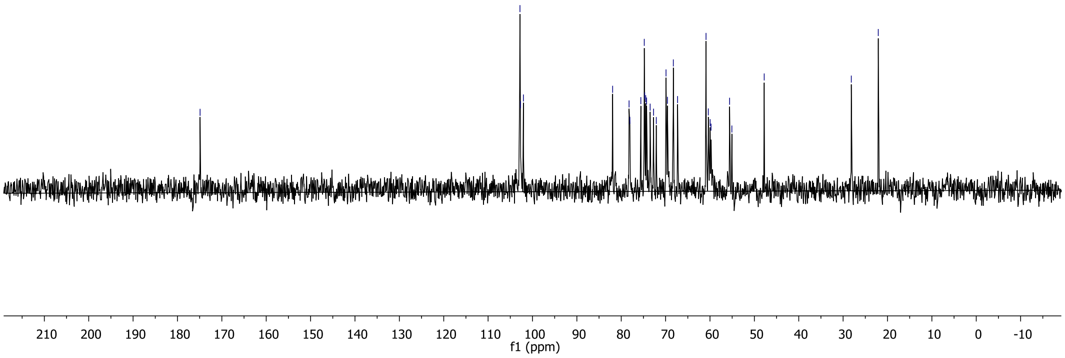
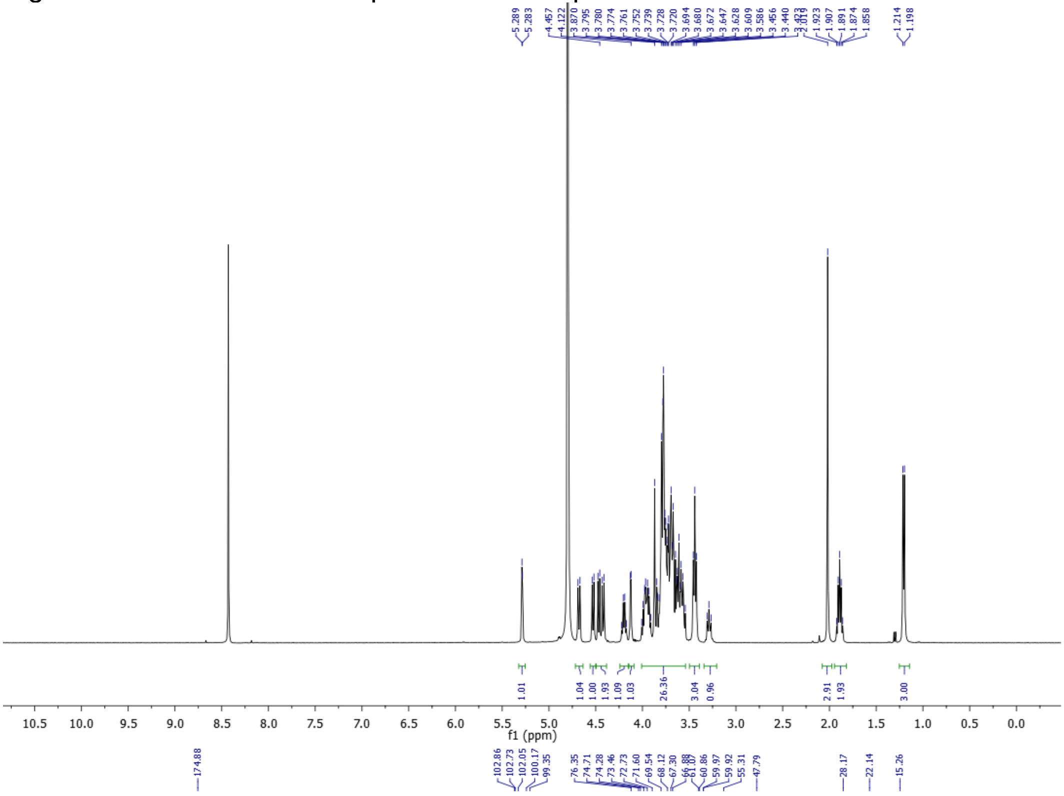


Figure S10 400 MHz NMR spectrum of compound **23** in D_2O at 300K



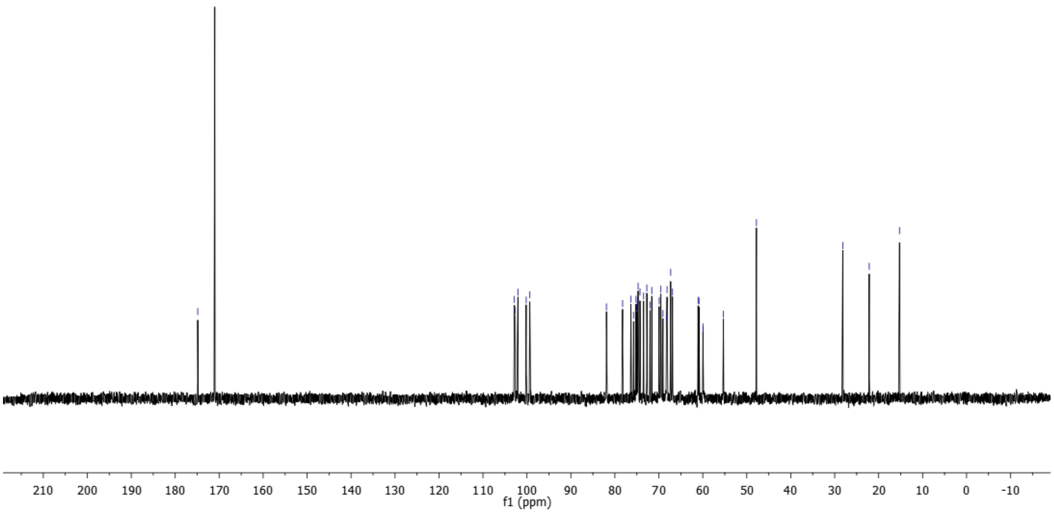


Figure S11 600 MHz NMR spectrum of compound **24** in D_2O at 300K

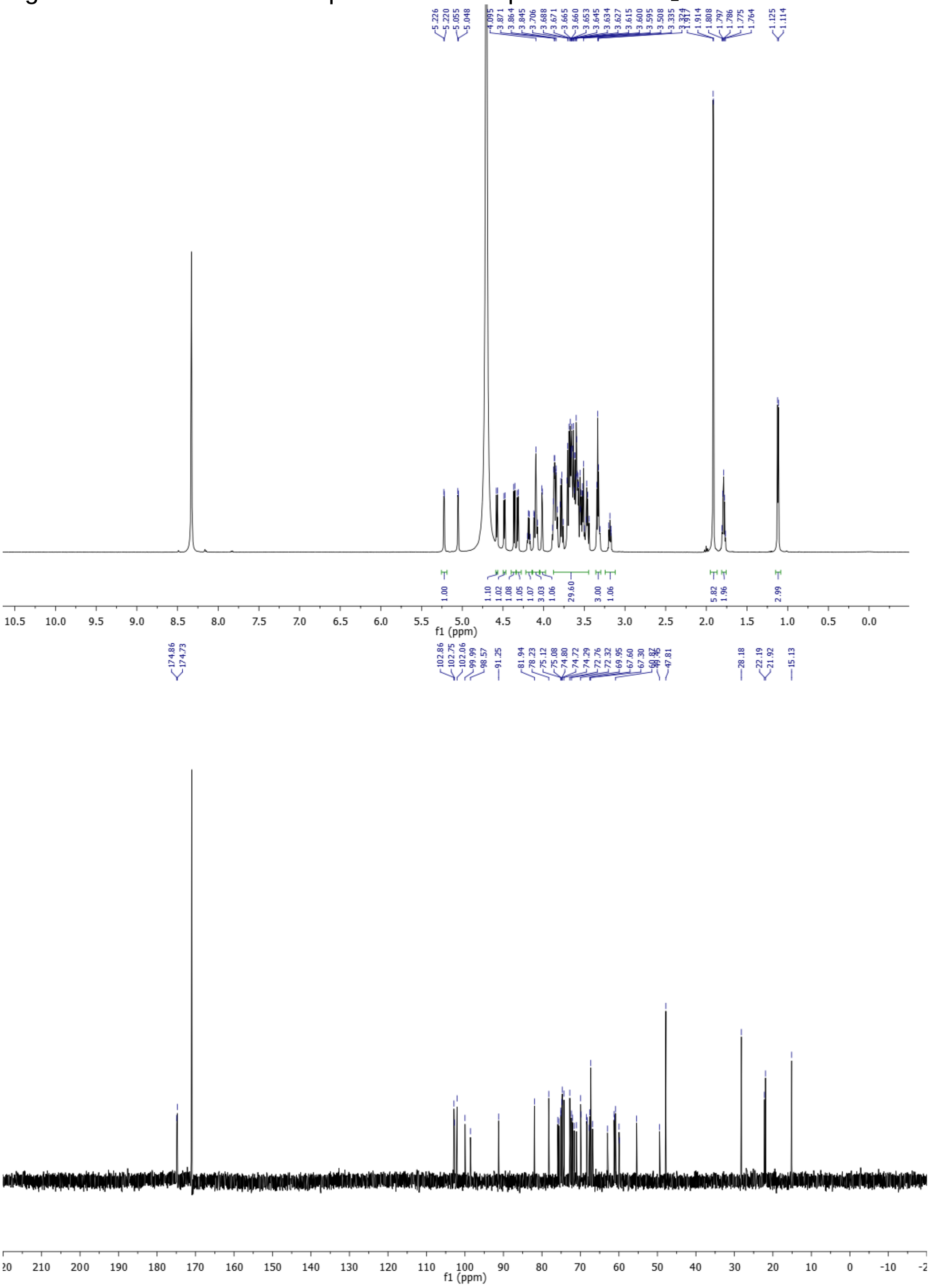


Figure S12 600 MHz NMR spectrum of compound **25** in D_2O at 300K

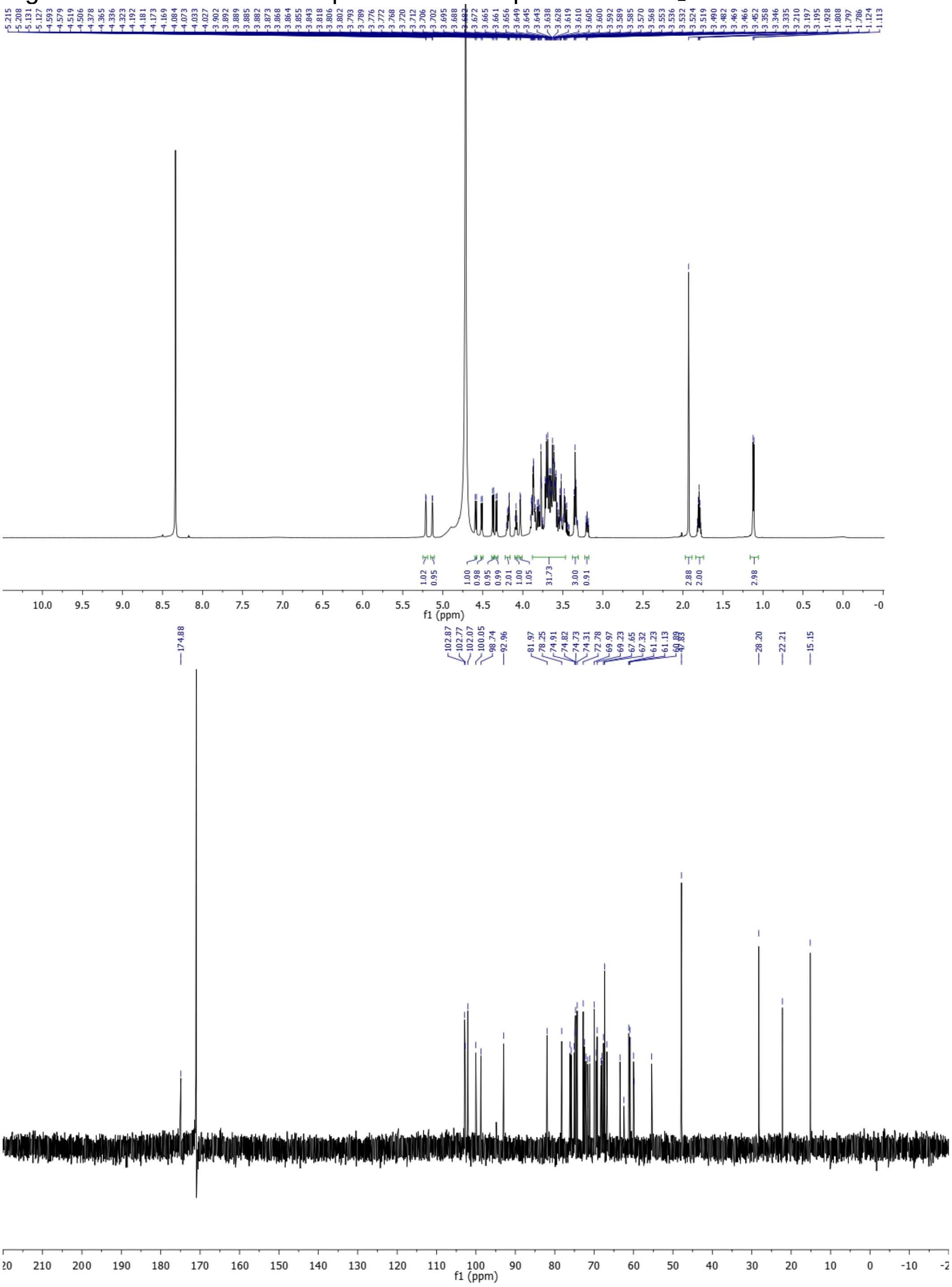


Figure S13 600 MHz NMR spectrum of compound **6** in D_2O at 300K

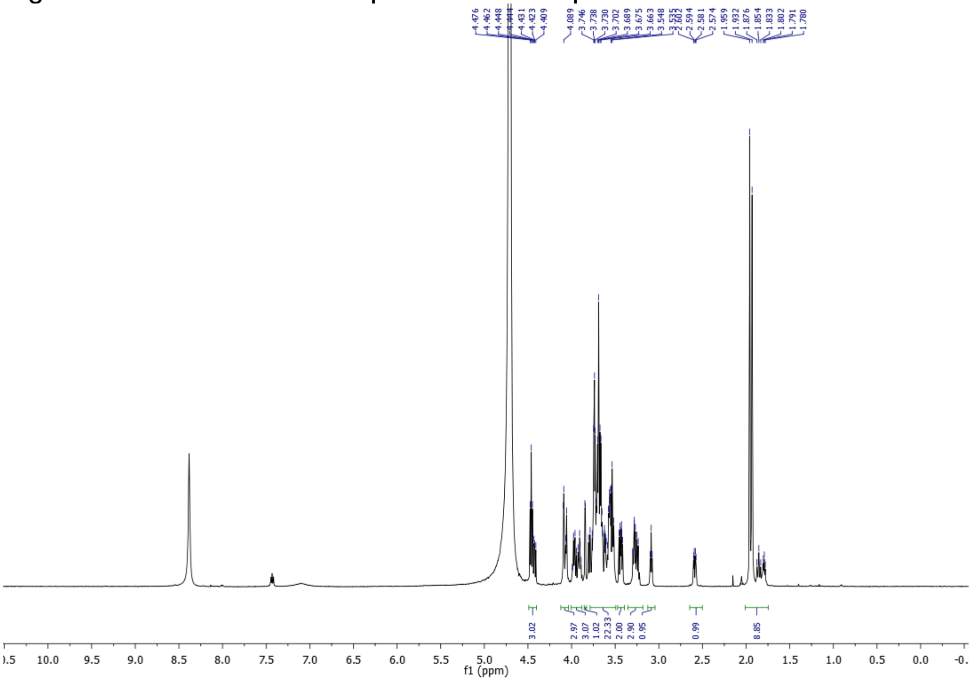


Figure S14 600 MHz NMR spectrum of compound **7** in D_2O at 300K

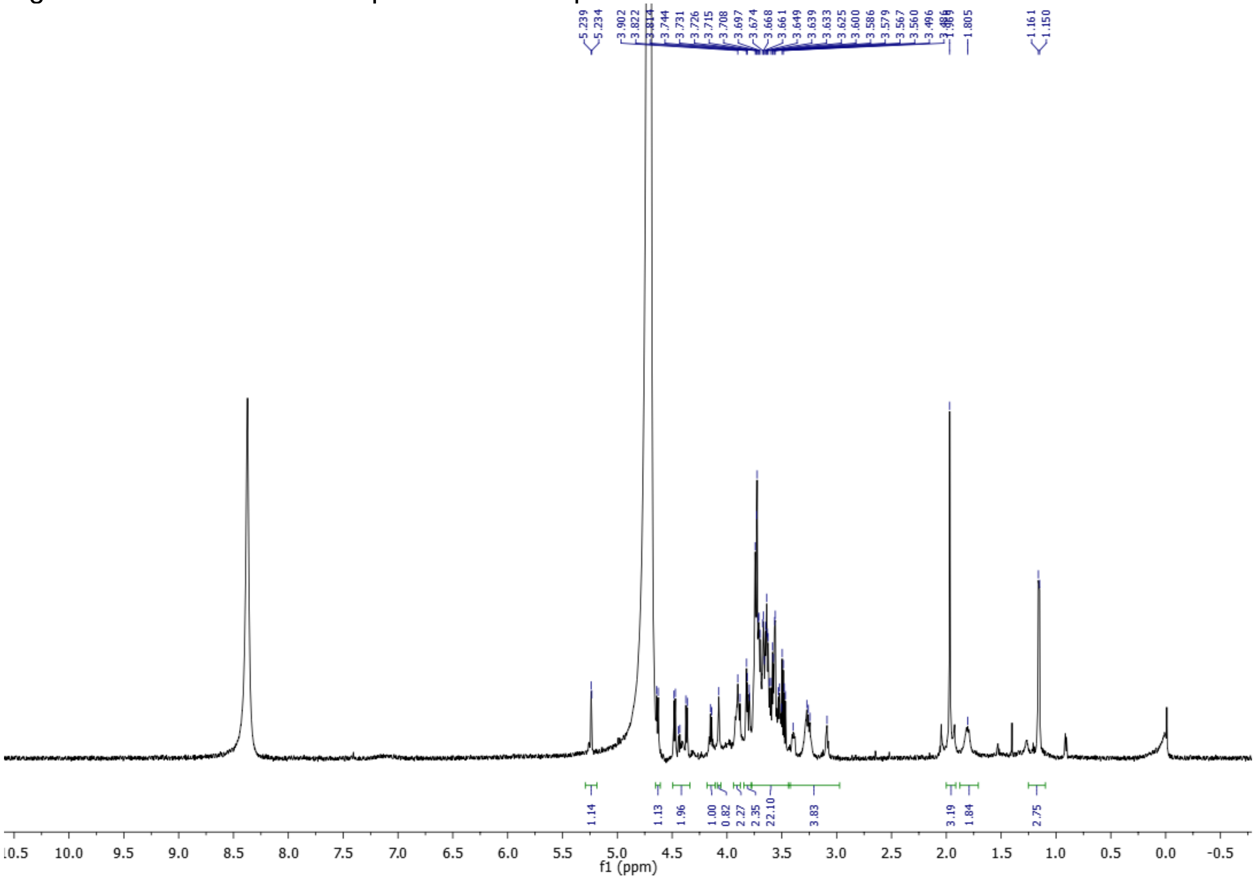


Figure S15 600 MHz NMR spectrum of compound **8** in D_2O at 300K

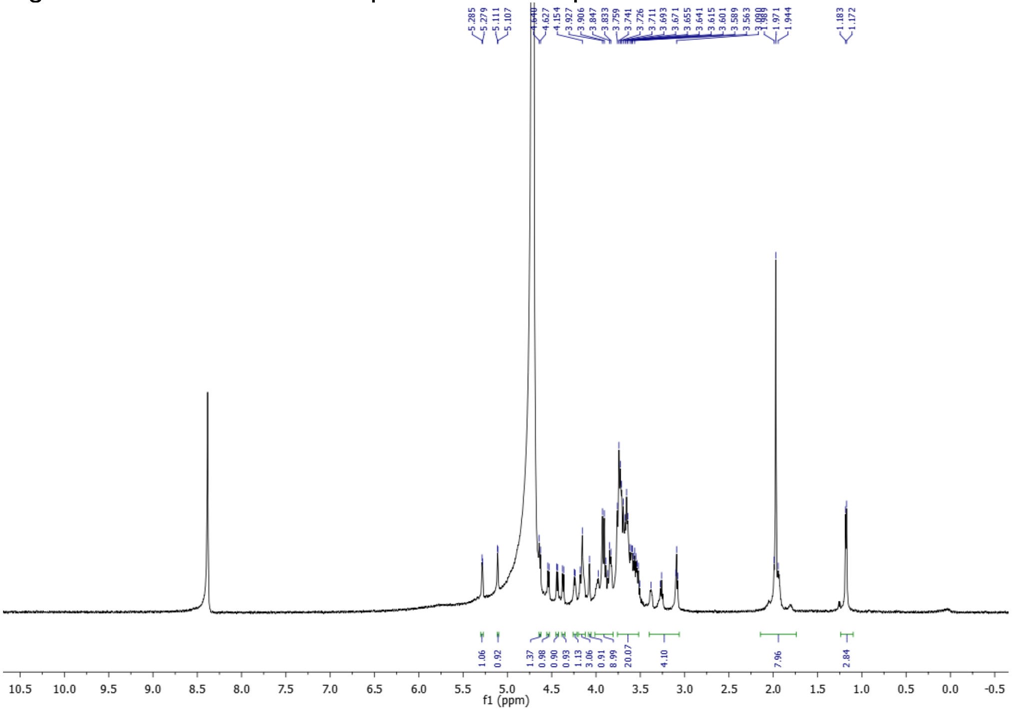
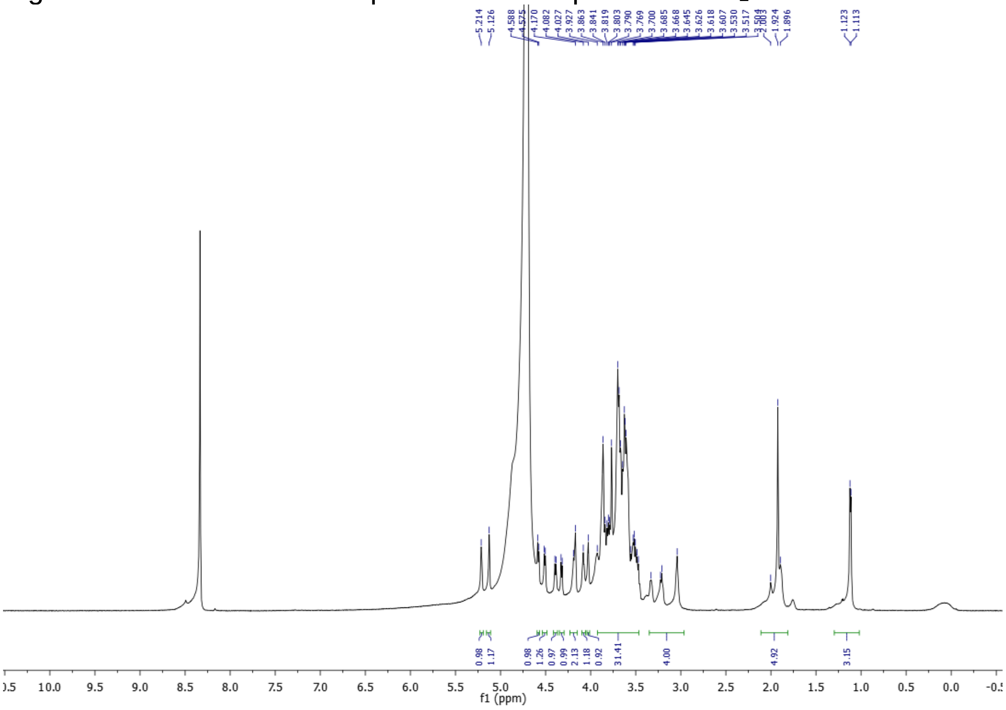


Figure S16 600 MHz NMR spectrum of compound **9** in D_2O at 300K



SUPPORTING INFORMATION

Figure S17 600 MHz NMR spectrum of compound **10** in D_2O at 300K

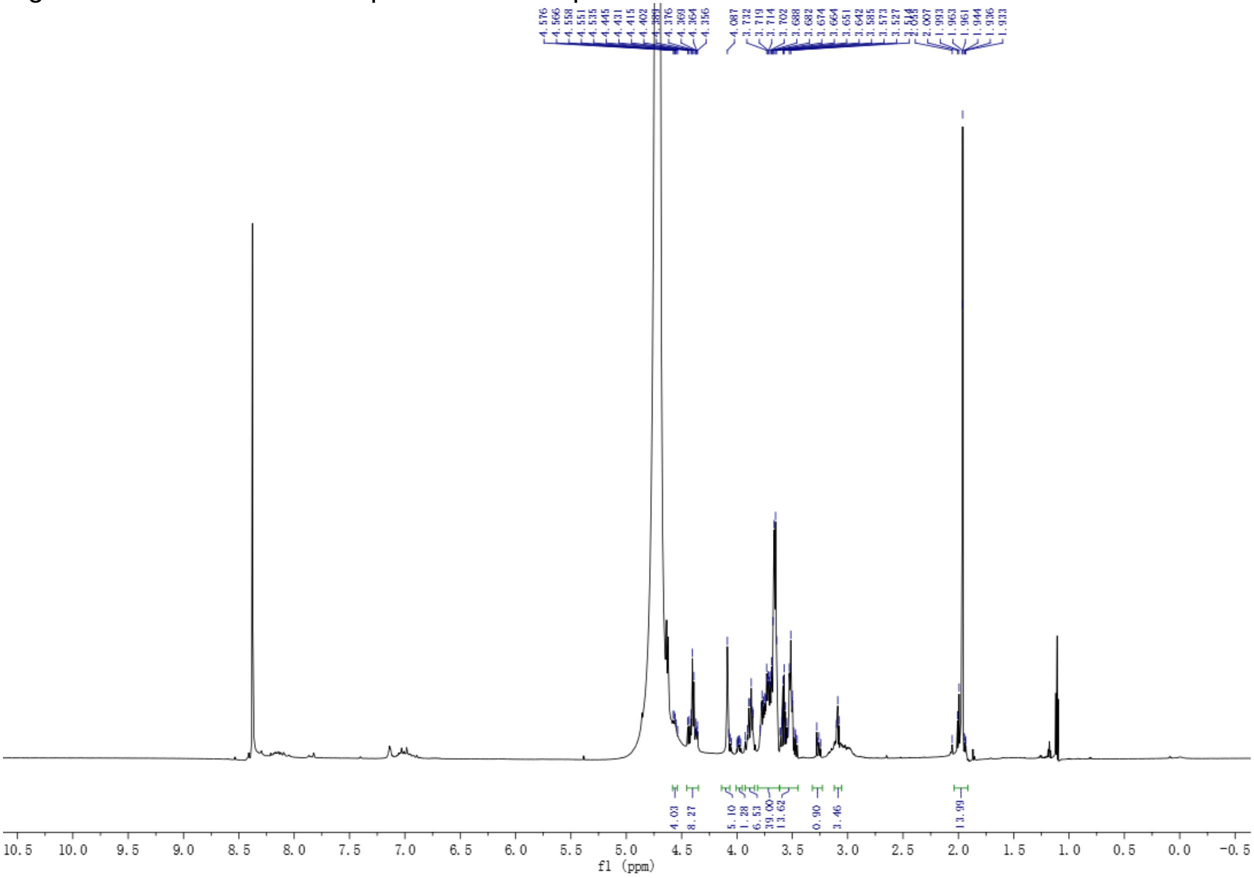


Table S1 Automated cycle

Step	Function	Command
1	Add reagent	Add sugar nucleotide buffer
2	Add reagent	Add enzyme buffer
3	Microwave method (glycosylation)	Microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s.
4	Microwave method (purification)	Microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s.
5	Add reagent	Add sugar nucleotide buffer
6	Add reagent	Add enzyme buffer
7	Microwave method (glycosylation)	Microwave 10 W, hold 6 h at 25°C, bubble on for 5 s, off for 600 s.
8	Microwave method (purification)	Microwave 50 W, hold 10 min at 90°C, bubble on for 1 s, off for 3 s, drain for 100 s.

References

[1] M. M. Muthana, J. Qu, Y. Li, L. Zhang, H. Yu, L. Ding, H. Malekan, X. Chen, *Chem. Commun. (Cambridge, England)* **2012**, *48*, 2728-2730.
[2] G. Zhao, W. Guan, L. Cai, P. G. Wang, *Nat. Proto.* **2010**, *5*, 636-646.
[3] Y. Li, M. Xue, X. Sheng, H. Yu, J. Zeng, V. Thon, Y. Chen, M. M. Muthana, P. G. Wang, X. Chen, *Bioorg. Med. Chem.* **2016**, *24*, 1696-1705.
[4] H. Yu, H. Yu, R. Karpel, X. Chen, *Bioorg. Med. Chem.* **2004**, *12*, 6427-6435.
[5] G. Sugiarto, K. Lau, J. Qu, Y. Li, S. Lim, S. Mu, J. B. Ames, A. J. Fisher, X. Chen, *ACS Chem. Biol.* **2012**, *7*, 1232-1240.
[6] a) L. Wen, Y. Zheng, K. Jiang, M. Zhang, S. M. Kondengaden, S. Li, K. Huang, J. Li, J. Song, P. G. Wang, *J. Am. Chem. Soc.* **2016**, *138*, 11473-11476; b) H. Yu, Y. Li, J. Zeng, V. Thon, D. M. Nguyen, T. Ly, H. Y. Kuang, A. Ngo, X. Chen, *J. Org. Chem.* **2016**, *81*, 10809-10824.
[7] H. Malekan, G. Fung, V. Thon, Z. Khedri, H. Yu, J. Qu, Y. Li, L. Ding, K. S. Lam, X. Chen, *Bioorg. Med. Chem.* **2013**, *21*, 4778-4785.
[8] S. E. Domino, L. Zhang, J. B. Lowe, *J. Biol. Chem.* **2001**, *276*, 23748-23756.
[9] W. Yi, J. Shen, G. Zhou, J. Li, P. G. Wang, *J. Am. Chem. Soc.* **2008**, *130*, 14420-14421.
[10] N. O. Seto, M. M. Palcic, O. Hindsgaul, D. R. Bundle, S. A. Narang, *Eur. J. Biochem.* **1995**, *234*, 323-328.
[11] C. Chen, Y. Zhang, M. Xue, X. W. Liu, Y. Li, X. Chen, P. G. Wang, F. Wang, H. Cao, *Chem. Commun. (Cambridge, England)* **2015**, *51*, 7689-7692.